European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

BAS 750F
(Mefentrifluconazole)

Volume 3 – B.9 (AS)

Rapporteur Member State: United Kingdom
Co-Rapporteur Member State: France & Austria

Version History

When	What
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B.9. ECOTOXICOLOGY DATA

This document supports the regulatory approval of BAS 750 F according to the approval criteria under 1107/2009 by evaluating the ecotoxicologically relevant data and performing risk assessments. This chapter provides the study summaries submitted in support of the first approval of the active substance BAS 750 F in Europe.

Furthermore a literature search was performed on the parent molecule and the discovered metabolites from the soil and aquatic compartments. But, being a new active substance, no references in the literature were found for BAS 750 F. Literature search was also extended to metabolites and checked for relevance in B.9.11.

According to Section 4 of 283/2013 and Section 5 of 284/2013 there is a need to ensure that there are appropriate methods of analysis, i.e. in Section 4.1.2 of 283/2013, it is stated that "methods shall be submitted, with a full description, for the determination of non-isotope-labelled residues in all areas of the dossier, as set out in detail in the following points: (f) in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies;"

The RMS has considered the method of analysis used for the ecotoxicological studies and the associated assessment is presented in III CA B.5.1.2.6 and IIICP B.5.1.2. In assessing these methods of analysis, several were deemed to comply with the criteria outlined in SANCO/3029/99 rev.4 and these are considered to be acceptable and hence can be used, without further consideration, for risk assessment purposes. In several instances, the following deficiencies were identified:

- *The suitability of matrix matched standards for calibration purposes has not been addressed*
- *Only 3 instead of 5 replicates have been used to determine recoveries and repeatability at each fortification level*
- *The linear range has not been demonstrated to be appropriate to the concentrations of the analyte in relevant analytical matrices*
- *In one method deficiencies in accuracy which may lead to an underestimation of the toxicity of BAS 750 F*

Presented below is a consideration of each of these deficiencies:

Linearity
SANCO/3029/99 rev.4 states that the analytical calibration should extend over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions ± at least 20%. In the validation data for some of the methods, the range does not cover the concentration of the analyte in the study. Whilst important, the RMS is of the view that this does not fundamentally undermine the validity of the studies concerned as when the method is used the samples can be diluted to bring them into the range of the calibration curve.

Repeatability
SANCO/3029/99 rev.4 states that five determinations should be made at each fortification level to determine relative standard deviation (RSD) of repeatability. In the validation data for some of the methods, only three determinations were made as opposed to five. The RMS is of the view that as the RSD in each case is below the acceptable limit of 20% given in the guidance that this provides sufficient confidence in the repeatability of the method. In the majority of the validation data at least two fortification levels were used, giving a total of at least six determinations, and the RSD for the six fortifications was also less than 20%, which supports the acceptability of the recovery.

Matrix matching
SANCO/3029/99 rev.4 states that when determining linearity the possible effects of sample components, e.g. co-extractives, on chromatographic transmission or detection system response must be addressed, and that where appropriate, detection system calibration should be generated using standard solutions in a matrix similar to that of the samples to be analysed. In the validation data for some of the methods to support ecotoxicology studies, linearity was determined using calibration (solvent) standards, rather than matrix matched standards. Where matrix matched standards are not used, this could result in suppression or enhancement of recovery, i.e. an over or under prediction of the actual residue level. In some circumstances other data may provide mitigation for the absence of matrix matched standards for a pre-registration method, however this depends on the nature of the method. The impact of the absence of matrix matched standards on the suitability of the methods of analysis is therefore considered on a case by case basis, taking into account the impact of inaccuracies in the determined residue levels on the risk assessment.

Deficiencies in accuracy
The method for measuring the recovery of BAS 750 F in one study (III CP B.9.7.1/3, Schulz L., 2015b) overestimates the level of the active in the soil cores based upon the recovery in the fortification level of 0.200 mg/kg being 151.08%, causing an underestimation of toxicity. This method is discussed further in III CP B.9.8.1.

Following consideration of additional data supplied by the applicant, it was considered that the methods of analysis used within ecotoxicological studies provided for BAS 750 F were sufficiently validated for the purposes of the regulatory process (III CP B.5.1.2).

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

Table B.9.1.1-1: Summary of Endpoints for birds

Organism	Test substance	Timescale (Test type)	Endpoint [a]	Toxicity value	Reference
Bobwhite quail (*Colinus virginianus*)	BAS 750 F	Single dose (Acute oral)	LD_{50} NOEL	**816 mg/kg bw** <300 mg/kg bw	B.9.1.1.1/1 [redacted] 2014a
Mallard duck (*Anas platyrhynchos*)	BAS 750 F	Single dose (Acute oral)	LD_{50} NOEL	**>2000 mg/kg bw** 2000 mg/kg bw	B.9.1.1.1/2 [redacted] 2014b
Canary (*Serinus canaria*)	BAS 750 F	Single dose (Acute oral)	LD_{50} NOEL	**>2860 mg/kg bw** <1001 mg/kg bw	B.9.1.1.1/3 [redacted] 2015a
Bobwhite quail (*Colinus virginianus*)	BAS 750 F	21 weeks (Sub-chronic and reproductive)	NOEC EC_{10} EC_{20}	285 mg a.s/kg diet **(25.3 mg a.s./kg bw/d)** ND ND	B.9.1.1.3/1 [redacted] 2014a
Mallard duck (*Anas platyrhynchos*)	BAS 750 F	20 weeks (Sub-chronic and reproductive)	NOEC EC_{10} EC_{20}	600 mg a.s/kg diet **(80.5 mg a.s./kg bw/d)** ND ND	B.9.1.1.3/2 [redacted] 2015a

Values in bold have been considered for the risk assessment

B.9.1.1.1. Acute oral toxicity to Birds

Report: B.9.1.1.1/1
[redacted] 2014a
BAS 750 F-Acute toxicity in the bobwhite quail (*Colinus virginianus*) after single oral administration (LD_{50})
2014/1095701
Guidelines: EPA 850.2100
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, CAS No.: 1417782-03-6, test substance number 11/0741-8, batch identification: COD-001740, purity 98.8% (tolerance ± 1.0%)

B. STUDY DESIGN

Test species: Bobwhite quail (*Colinus virginianus*) before their first egg-laying season, visually indistinguishable from wild birds. Supplied by Wachtelzucht Küberich GbR, Geesdorf-Wiesentheid, Germany.

Age: Approximately 22 weeks old at dosing, hatch date: 26 Apr 2013.

Test design: Birds were administered single doses of the test substance BAS 750 F undiluted in 2 gelatine capsules per animal directly into the crop following 14 days acclimation. The control group was dosed with two empty capsules per animal. 5 males and 5 females per dose group were used. The birds were observed for regurgitation at least for 1 hour after dosing. An observation period of 14 days followed, during which mortalities and signs of toxicity were recorded, four times on the day of dosing and daily thereafter.

Housing: Stainless steel cages with Noryl® floor, cardboard and a sand bath. Area of 1.3m^2 for 5 test organisms.

Organism examinations: Individual body weights were determined 1 day prior to dosing and group mean body weights calculated on the day of dosing and on days 7 and 14 after dosing. Mean food consumption (g/bird/day) was calculated from the weekly food consumption/cage separately for male and female birds for the first and second week after dosing. Wasted food and excessive spills from cages was recorded. A gross post-mortem examination was conducted for all birds that died during the study and all birds sacrificed by CO_2 asphyxia at the termination of the observation period.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations. Calculation of LD_{50} and NOEL.

Test concentrations: 0 (Control), 300, 480, 770, 1250 and 2000 mg a.s./kg body weight, all corrected for the a.s. purity

Test conditions: Birds fasted for 17-19 hours before administration of the test substance;

Temperature: 17.9°C (minimum) and 21.8°C (maximum); time below limits (21°C ± 2°C) for 13 hours;

Relative humidity: 44.9-100%. Time below and above limits of 45-70% was 15 minutes and 15 days 12 hours and 30 minutes respectively.

The deviations were considered to have no relevant influence on the test results, since the appearance and behaviour of the birds in the control group was normal and did not indicate any adverse effects.

10-fold air exchanges/hour;

Photoperiod: 8 hours light, 16 hours dark, warm-light fluorescent lamps. Light intensity (floor in the middle of the cages): 21-90lux.

Statistics: Statistical calculation of the LD_{50} was performed using probit-analysis according to Finney and the NOEL was calculated with Fisher´s exact test (one-sided), calculated using SAS system. Body weight was compared using Dunnet test (two-sided), calculated using ToxData PDS Ltd.

II. RESULTS AND DISCUSSION

All the acceptability criteria were met (EPA 850.2100 (1996)):

- ≤10% control bird mortality (0%)

Biological results are summarised in Tables B.9.1.1.1-1 and 8.1.1.1-2 below. No regurgitation was observed during the first hour after dosing. Highest dose tested causing no mortality was 300 mg a.s./kg b.w. for males and 480 a.s./kg b.w. for females. The acute oral LD_{50} value was 816 mg a.s./kg b.w. (95% confidence interval: 640 –1038 mg active substance/kg body weight).

Toxic signs were observed in all dose groups. In the 300 mg a.s./kg b.w. dose group, diarrhoea was observed until day 3 after dosing, most likely as a consequence of the reduced food uptake in the first week after dosing. In the 480 mg a.s./kg b.w. dose group, diarrhoea was observed until day 5 after dosing for the same reason as in the lowest dose group. In one male, apathy was observed before it died and slight apathy was observed in 4 females. In the 770 mg a.s./kg b.w. dose group, diarrhoea was observed until day 7 after dosing. Apathy was observed in all males and 3 females. In the 1250 mg a.s./kg b.w. dose group, diarrhoea was observed until day 10 after dosing. Apathy was observed in all males and 3 females. One male bird was killed in extremis. In the 2000 mg a.s./kg b.w. dose group, diarrhoea was observed until all birds died. Apathy was observed in all males and 3 females.

Substance-related impairment of food uptake in comparison to the control was observed in all of the dose groups. In the first week after dosing the food consumption was decreased in all dose groups. The decrease was dose dependent. In the second week after dosing, the food consumption of the surviving birds returned to values which were similar to or higher than the food consumption of the control group, indicating a compensation effect, except for the surviving male animal of the dose group 1250 mg a.s./kg b.w.

Table B.9.1.1.1/1-1: Dietary intake of the northern bobwhite (*Colinus virginianus*) following

Day	Dose rate [mg a.s./kg b.w.]					
	0 (control)	300	480	770	1250	2000
Male						
0-7	12.7	9.4	7.6	4.0	2.3	1.1
7-14	13.2	13.9	14.8	15.4	7.1	-
Mean	13.0	11.7	11.2	9.7	4.7	-
Female						
0-7	11.0	8.9	7.2	2.3	2.9	1.0
7-14	10.5	14.2	14.0	14.1	14.5	-
Mean	10.8	11.6	10.6	8.2	8.7	-

- All test organisms dead

Substance-related reduction of the body weights in the male and female birds was observed in the first week in all of the dose groups compared to the control with a dose-related trend. The deviation was statistically significantly different compared to the control group in dose groups ≥480 mg active substance/kg body weight.

Two weighing errors occurred. One female bird that weighed 114.9g at day -14 when randomisation occurred weighed 198.0g on day -1. Consequently the birds has to be reassigned resulting in the 300mg/kg bw dose having a higher mean female bodyweight than the other groups, although the increase was not significant. Erroneously one female bird in the control group was not weighed on day 7. Given the body weight of the bird was in line with the others on days -1 and 14, this error is not expected to have influenced the LD_{50} calculation.

Table B.9.1.1.1/1-2: Body weight of the northern bobwhite (*Colinus virginianus*)

Day	Dose rate [mg a.s./kg b.w.] (standard deviation)					
	0 (control)	300	480	770	1250	2000
Male						
-1	179.5 (13.0)	190.6 (9.9)	182.3 (12.2)	184.0 (8.1)	187.7 (5.0)	186.6 (13.4)
7	179.2 (11.8)	176.4 (13.3)	157.5 (11.7)	139.5* (21.0)	155.4 (22.8) [a]	105.7 (0.8) [a]
14	188.3 (10.2)	191.3 (12.9)	178.2 (10.4)	173.7 (19.1)	153.6 [a]	-
Female						
-1	183.8 (5.9)	191.6 (5.1)	184.3 (7.0)	180.0 (11.9)	180.4 (13.5)	184.1 (7.6)
7	178.2 (4.9)	180.2 (6.3)	157.3* (16.7)	145.5[a]	140.9 (39.8) [a]	-
14	183.4 (8.5)	188.8 (5.1)	179.0 (7.2)	153.3 [a]	166.1 (29.8) [a]	-

[a] Excluded from statistical analyses due to only one or two birds surviving
*Significantly different from the control

Seven birds that died had liquid content in the gut (dose groups ≥480 mg active substance/kg body weight). In birds sacrificed at the end of the study, no abnormalities were observed. The relevant data

and endpoints are summarised in the table below.

Table B.9.1.1.1/1-2: Acute toxicity of BAS 750 F to the northern bobwhite (*Colinus virginianus*)

	Dose rate [mg a.s./kg b.w.]					
	0 (control)	300	480	770	1250	2000
Number of birds per dose group	10	10	10	10	10	10
Number of dead birds	0	0	1	6	7	10
Dead birds percentage [%]	0	0	10	60	70	100

Endpoints	Dose [mg a.s./kg b.w.]
Highest dose causing no substance-related mortality	300
LD_{50} (14 d)	816
NOEL[1)]	480

b.w.=body weight
1) NOEL is the highest tested concentration without significant effects

III. CONCLUSION

The acute oral median lethal toxicity (LD_{50}) of BAS 750 F was 816 mg active substance/kg body weight. The "No Observed Effect Level" (NOEL) for mortality was 480 mg active substance/kg body weight. The NOEL for toxic effects was <300 mg a.s./kg bw.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an LD_{50} of 816 mg a.s./kg bw and a NOEL for toxic effects of <300 mg a.s./kg bw. The RMS agrees that the test conditions being outside the guideline levels did not have a negative impact on the test.

The agreed endpoint suitable for use in the risk assessment is:
LD_{50} of 816 mg a.s./kg bw

Report: B.9.1.1.1/2
[redacted] 2014b
BAS 750 F-Acute toxicity in the mallard duck (*Anas platyrhynchos*) after single oral administration (LD_{50})
2014/1095700
Guidelines: EPA 850.2100, EPA 850.2000
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, CAS No.: 1417782-03-6, test substance number 11/0741-8, batch identification: COD-001740, purity 98.8% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species:	Mallard duck (*Anas platyrhynchos*) before their first egg-laying season, visually indistinguishable from wild birds. Supplied by Deindl GmbH & Co. KG, Geflügelzucht und –vertrieb, Rietberg, Germany.
Age:	Approximately 24 weeks old at dosing, hatch date: 13 Apr 2013.
Test design:	5 males and 5 females per dose group were administered a single dose of 2000 mg a.s./kg b.w undiluted in 4 gelatine capsules per animal directly into the crop. The control group was dosed with four empty capsules per animal. The birds were observed for regurgitation at least for 1 hour after dosing. An observation period of 14 days followed, during which mortalities and signs of toxicity were recorded, four times on day of dosing and daily thereafter.
Housing:	Steel wire pens (1.3×0.95×1.3m) with steel mesh floors covered with plastic mats. Area of 1.235m^2 for 5 test organisms.
Organism examinations:	Individual body weights were determined and the group means calculated separately for male and female birds on the day before dosing and on days 7 and 14 after dosing. Mean food consumption (g/bird/day) was calculated from the weekly food consumption/cage separately for male and female birds for the first and second week after dosing. The cages were also checked for wasted food and excessive spills were recorded. A gross post-mortem examination was conducted for all birds that died during the study and all birds sacrificed by CO_2 asphyxia at the termination of the observation period. The examination included the GI tract, liver, kidney, heart, reproductive organs, spleen, subcutaneous fat, skeletal muscles and thyroid glands.
Endpoints:	Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations. Calculation of LD_{50} and NOEL.
Test concentrations:	0 (control) and 2000 mg a.s./kg body weight
Test conditions:	Birds fasted for about 17-18 hours before administration of the test substance;
	Temperature: 16.4°C (minimum) and 19.8°C (maximum); time below limits (21±2°C) was 8 hours during exposure and 13d, 23h and 15 min before exposure, no time above limits;
	Relative humidity: 51.8% (minimum) and 100.0% (maximum), no time below limits, time above limits was 6 hours during exposure and 25d, 11h and 30min before exposure.
	The deviations were considered to have no relevant influence on the study results, since the birds were apparently healthy during the whole acclimation and observation period;
	10-fold air exchanges/hour;
	Photoperiod: 8 hours light, 16 hours dark, warm-light fluorescent

lamps. Light intensity (floor in the middle of the cages): 59-66lux.

Statistics: No statistical calculation of the LD_{50} was performed since no mortality was observed in the tested dose and the NOEL was calculated with Fisher´s exact test calculated with SAS System. Body weight was compared with Dunnett test (two-sided) calculated with ToxData® of the PDS Ltd.

II. RESULTS AND DISCUSSION

All the acceptability criteria were met (EPA 850.2100 (1996)):

- ≤10% control bird mortality (0%)

Biological results are summarised in Table8.1.1.2-1 below. Highest dose tested causing no mortality was 2000 mg a.s./kg b.w.. The following acute oral LD_{50} value of the test substance in mallard duck was determined at the end of the observation period: LD_{50} >2000 mg a.s./kg b.w. No regurgitation was observed after dosing.

No toxic signs were observed in the control or in the dose group. Highest dose tested without toxic signs: 2000 mg active substance/kg b.w.

The body weight was not statistically significantly reduced in the male and female birds at day 7 or at day 14 (sacrifice) in the dose group and the body weight development was not impaired in comparison to the control group.

No abnormalities were observed during gross post-mortem examinations.

Table B.9.1.1.1/2-1: Acute toxicity of BAS 750 F to the mallard duck (*Anas platyrhynchos*)

	Dose rate [mg a.s./kg b.w.]	
	0 (control)	2000
Number of birds per dose group	10	10
Number of dead birds	0	0
Dead birds percentage [%]	0	0

Endpoints	Dose [mg a.s./kg b.w.]
Highest dose causing no substance-related mortality	2000
LD_{50} (14 d)	> 2000
NOEL[1)]	2000

b.w.=body weight

1) NOEL is the highest tested concentration without significant substance related effects

III. CONCLUSION

The acute oral median lethal toxicity (LD_{50}) of BAS 750 F was >2000 mg active substance/kg body weight. The "No Observed Effect Level" (NOEL) was 2000 mg active substance/kg body weight.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an LD_{50} of >2000 mg a.s./kg bw and a NOEL for mortality of 2000 mg a.s./kg bw. The RMS agrees that the environmental test conditions being outside the guideline levels did not have a negative impact on the test, and that the temperature exceeded self-imposed rather than the guideline limits, whereas maximum humidity of 100% exceeded the guideline maximum of 70% . Additionally it is noted that the variation in the test organisms' body weight of 16.8% exceeds the recommended ±10% in EPA 850.2100 (1996), although as no negative effects were observed in the controls and consistent responses were observed across test organisms, this deviation is not considered to have adversely affected the test.

The agreed endpoint suitable for use in the risk assessment is:
LD_{50} of >2000 mg a.s./kg bw

Report:	B.9.1.1.1/3 [redacted] 2015a BAS 750 F-Acute toxicity in the Canary (*Serinus canaria*) after single oral administration (LD_{50}) 2015/1085493
Guidelines:	EPA 850.2100
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch identification: COD-001740, purity 98.8% (tolerance ± 1.0%),

B. STUDY DESIGN

Test species: Canary (*Serinus canaria*) before their first egg-laying season, phenotypically indistinguishable from wild birds. Supplier: Zoowelt – Welt der Tiere, Bechtheim, Germany

Age of the test animals: 18 weeks at dosing, hatch date: 21 to 27 July 2014

Test design: Birds were administered doses of the BAS 750 F undiluted in soluble hard gelatine capsules directly into the crop after a fastening time of 2 to 3 hours. 3 capsules per animal were used; 5 males and 5 females housed separately per dose group received the treatment. The control groups received the same amount of untreated capsules. The birds were observed for regurgitation for 1 to 2 hours after dosing. An observation period of 14 days followed, during which mortalities and signs of toxicity were recorded. Mortality and clinical signs checks were made before dosing, about 1, 2 and 4 hours after dosing, and daily thereafter.

Housing: Steel wire pens (1.67×0.76×0.75m) with steel floors covered with cardboard, a water bath, perch, climbing tree, 2 feed hoppers, water supply and a limestone. Area of 1.235m^2 per 5 test organisms.

Organism examinations: Determination of individual body weights and calculation of the group means separately for male and female animals on day -14 (for randomization), on the day before dosing and on days 7 and 14 after

dosing. Mean food consumption (g/animals/day) was calculated for males and females separately every two days by cage. A gross post-mortem examination was conducted for all birds that died during the study and all animals sacrificed by CO_2 asphyxia at test termination. The examination included the GI tract, liver, kidney, heart, reproductive organs, spleen, subcutaneous fat, skeletal muscles and thyroid glands.

Endpoints: Mortality, clinical observation, food consumption, body weight (b.w.), and gross-pathological examinations. Calculation of LD_{50} and NOEL.

Test concentrations: 0 (control), 1001, 1302, 1692, 2200 and 2860 mg a.s./kg b.w.

Test conditions: The birds were fasted for 2 to 3 hours before administration of the test substance.

Temperature: 21.0°C (minimum) and 22.3°C (maximum); no time above/below the limits;

Relative humidity: 41.8% (minimum) and 63.2% (maximum), no time above the limit, 2 days were below the limit of 45%.

10-fold air exchanges/hour;

Photoperiod: 10 hours light, 14 hours dark, warm-light fluorescent lamps. Light intensity (floor in the middle of the cages): 89-98lux.

Statistics: No statistical calculation of the LD_{50} was performed since no dose response for mortality was observed in the tested doses. The NOEL was calculated using Fisher's exact test (one sided). For body weight and body weight change, Dunnett-test (two-sided) was used to compare dose groups with the control group.

II. RESULTS AND DISCUSSION

All the acceptability criteria were met (EPA 850.2100 (1996)):

- ≤10% control bird mortality (0%)

Diarrhoea on the day of dosing was considered to be a consequence of the fasting period and was observed in the dose groups as well as in the control group. Therefore, it was not considered to be a substance-related effect to the same extent. Apathy was observed in all dose groups, and is presented in Table B.9.1.1.1/3-1 below. No toxic signs were observed in the control.

No marked substance-related impairment of feed uptake in comparison to the control was observed in any of the tested dose groups. The body weight was not statistically significantly reduced in the male and female birds at day 7 or at day 14 (sacrifice) in the tested dose group and the body weight development was not impaired in comparison to the control group.

One female animal of the lowest dose group (1001 mg a.s./kg b.w.) died one day after dosing, as well as one female animal in the highest dose group (2860 mg a.s./kg b.w). The mortality did not exceed 10% and no further mortality was observed in any other group. As the mortalities cannot be attributed to the test substance there was no dose-response relationship in the findings. Hence, the LD_{50} calculation by Probit analysis was not performed and the acute oral LD_{50} value >2860 mg a.s./kg b.w.

No substance-related findings were observed during the gross post-mortem examination. In one female of the dose group 1001 mg a.s./kg b.w., a liquid content of the rectum was observed.

Table B.9.1.1.1/3-1: Acute toxicity of BAS 750 F to the canary (*Serinus canaria*)

	Dose rate [mg a.s./kg b.w.]					
	0 (control)	1001	1302	1692	2200	2860
Number of birds per dose group	10	10	10	10	10	10
Number of apathetic birds	0	1 male 3 female	5 female	5 female	2 male 5 female	5 female
Apathy duration (days)	-	<1	<1	1	2 <1	1
Number of dead birds	0	1	0	0	0	1
Dead birds percentage [%]	0	10	0	0	0	10
Endpoints	**mg a.s./kg b.w.**					
Highest dose causing no substance-related mortality	2860					
LD_{50} (14 d)	> 2860					
NOEL[1)]	<1001					

b.w.=body weight
1) NOEL is the highest tested concentration without significant substance related effects

III. CONCLUSION

The acute oral median lethal toxicity (LD_{50}) of BAS 750 F was >2860 mg a.s./ kg b.w. The "No Observed Effect Level" (NOEL) was <1001 mg active substance/kg body weight.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an LD_{50} of >2860 mg a.s./kg bw and a NOEL for mortality of ≥2860 mg a.s./kg bw. The RMS agrees that humidity exceeding the guideline levels did not have a negative impact on the test, and that the temperature passed outside self-imposed rather than guideline limits. It is additionally noted that food should be withheld for a minimum of 15h (EPA 850.2100 (1996) prior to dosing rather than 2-3h, and the variation in body weight was 12.3%, only marginally exceeding the recommended maximum of 10%. Given that the capsules were ingested and no regurgitation was observed, these deviations are not expected to have adversely affected the experiment.

The agreed endpoint suitable for use in the risk assessment is:
LD_{50} of >2860 mg a.s./kg bw

B.9.1.1.2. Short-term dietary toxicity to birds

No short-term oral toxicity studies in the mammalian toxicology section (B.6.3) indicate a potential for the dietary LD_{50} measured by the short- term dietary toxicity study to be lower than the LD_{50} based on an acute oral study Therefore short-term dietary toxicity to bird studies are not a data requirement as per Commission Regulation (EU) No 283-2013.

Report:	B.9.1.1.2/1 [redacted] 2014c BAS 750 F-Avian dietary toxicity test in chicks of the bobwhite quail (*Colinus virginianus*) 2014/1127963
Guidelines:	EPA 850.2200, OECD 205
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Bobwhite Quail (*Colinus virginianus*), chicks, hatched from eggs of animals indistinguishable from wild birds.

Age: 13 days old at start of substance feeding;

Source: Wachtelzucht Küberich GbR Geesdorf/Wiesentheid, Germany.

Test design: The test substance was administered via the diet for 5 days to 13-day old northern bobwhite quails. The birds were not sexed. 10 animals per dose group were used; dietary exposure period of 5 days plus a post exposure observation period of 6 days. Assessment of mortality and signs of clinical toxicity was carried out four times on day of dosing and daily thereafter.

Housing: Steel wire pens (1.3×0.65×1.3m) with wire mesh floors partially covered with a plastic mat, a food hopper, sand bath, toys and containers with drinking water and hay. Area of 0.845m^2 for 10 test organisms.

Organism examinations: Assessment of body weight was carried out on days 0, 5, 8 and 11. Food consumption was determined per cage daily. A gross post-mortem examination was conducted for all birds that died during the study and all animals sacrificed by CO_2 asphyxia at the termination of the study. The examination included the GI tract, liver, kidney, heart, reproductive organs, spleen, subcutaneous fat, and skeletal muscles.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations. Calculation of LC_{50}, LDD_{50} and NOEL.

Test concentrations: 0 (Control), 1480, 2222, 3333, 5000 and 7500 mg a.s./kg (nominal concentration based on active substance/kg diet).

Test conditions: Chicks were administered treated feed for 5 consecutive days followed by a post-exposure period of six days basal diet ad libitum without test substance;

Temperature: 19.7°C – 23.6°C;

Relative humidity: 34.2-100%;

Photoperiod: 14 hours light, 10 hours dark, light intensity 51-58lux in the middle of the cages on the floor level, warm light fluorescent lamps.

Analytics: The test substance concentrations were analysed using HPLC. The limit of quantification was 10ppm.

Statistics: LC_{50} and LDD_{50} Calculation: Probit-analysis according to Finney and Goodness-of-Fit-Test (Pearson Chi-Square); NOEL calculation: Fisher's exact test (one-sided); comparison of b.w.: Dunnett test (two-sided). SAS System was used to perform statistical analyses. The daily dose for each concentration group was calculated according to the following formula:

$$\text{Daily dose (mg/kg b.w./day)} = \frac{\text{Uptake test substance (mg/bird/day)} * 1000 \text{ (g/kg)}}{\text{Mean body weight of day 0 and day 5 (g/bird)}}$$

II. RESULTS AND DISCUSSION

All the validity criteria were met (EPA 850.2200(2012)):

- Random bird assignment
- ≤10% control mortality or moribund birds (0%)
- Maintenance of concentrations within ±20% of the nominal (97-114%)
- 5 day test substance administration (5d)
- ≥10 birds for each test concentration (10)
- Test substance administered within the diet
- ≥5 test substance concentrations and controls (5 and a control)

The concentration analyses in the feed yielded concentrations in a range of 97-114% of the nominal concentrations. Therefore the substance was maintained within ±20% of the nominal and endpoints can be based upon nominal values.

Substance-related mortality was observed in test concentrations ≥ 3333 mg active substance/kg diet. No test concentration led to 100% mortality. The LC_{50} was 6377 mg active substance/kg diet (95% confidence interval: 4909 – 12086 mg active substance/kg diet). The LDD_{50} calculated on the basis of daily doses was 858 mg a.s./kg b.w./day. The mortality rate was not correlated with the daily dose, potentially due to the effect of starvation influencing toxicity. No clinical signs of toxicity were observed in the control group and concentration groups ≤5000 mg a.s./kg b.w./day.

During the 5-day exposure period, the food consumption was decreased in all tested concentration groups. The decrease in food consumption was dose dependent, although for the three lower test concentrations the decrease was similar and not correlated to the dose. Post-exposure, in the highest concentration group, the food consumption of the surviving birds was 112% of the value of the control group, a possible indicator of compensation. For all other concentrations food consumption was lower (62-79%) than that of the control group

The body weights of the surviving chicks in all treated groups were statistically significantly decreased at the end of the exposure period on day 5. Body weight appeared to be dose dependent. At the end of the 6 day post-exposure period the body weights of the groups exposed to 3333 and 5000 mg a.s./kg diet were still statistically significantly different from the control. The body weight development during the exposure period was statistically significantly decreased in comparison to the control group in all tested concentration groups. During the post exposure period, the body weight development in the surviving chicks was similar to the control group and even statistically significantly increased during days 8 and 11 in the highest concentration group receiving 7500 mg a.s./kg diet. In all tested concentration groups the body weight development indicated a recovery after the end of exposure. No substance-related macroscopic abnormalities were detected in the gross-pathological post-mortem examination.

Table B.9.1.1.2/1-1: Avian dietary toxicity of BAS 750 F to the bobwhite quail (*Colinus virginianus*)

Parameter	Dose groups [mg a.s./kg diet]					
	Control	**1480**	**2222**	**3333**	**5000**	**7500**
Mortality [dead/survivor]	0/10	0/10	0/10	3/10	1/10[1)]	7/10
Daily dose [mg a.s./kg b.w./d] [4)]	not applicable	221	439	650	769	653
Mean feed consumption during (exposure) days 1 to 5 [g feed/bird/day]	6.8	3.8	4.7	4.4	3.2	2.0
Mean body weight on day 0 and day 5 [g/bird] [2)3)]	24.8/33.6	23.5/28.1**	25.1/27.3**	24.0/23.1**	24.3/21.4**	24.9/21.8**
Clinical signs	n.d.	n.d.	n.d.	n.d.	n.d.	a, t in 1 chick
Endpoints [mg a.s./kg diet]						
LC_{50}	6377					
NOEL (mortality)	<1480					
Endpoints [mg a.s./kg b.w./day]						
LD_{50}	858					
NOEL (mortality)	<221					

a.s. = active substance
b.w. = body weight
n.d. = no symptoms detected
a = apathy
t = tumbling

1) One chick died in the post-exposure period.
2) Statistic Profile=Dunnett test (two-sided), * $p \leq 0.05$, ** $p \leq 0.01$
3) Statistical analysis did not reveal significant differences
4) Based on measured concentration of active substance in the diet

III. CONCLUSION

Under the conditions of this study the LC_{50} for chicks of the bobwhite quail (*Colinus virginianus*) was 6377 mg active substance/kg diet. The LDD_{50} calculated on the basis of daily doses was 858 mg a.s./kg b.w./day. The NOEL calculated on the basis of daily dose was <221 mg/kg b.w. /day.

RMS Comment: The RMS notes that food avoidance and the toxic effect of the item has impacted food consumption and bodyweight data. Consequently, higher concentration dose groups resulted in

reduced daily doses. Therefore, despite some clear effects on mortality, the effects cannot be separated from the issue of food avoidance meaning doses cannot reliably attributed to effects. Additionally, the effect of food avoidance may be causing starvation and contributing to the mortalities. These issues will result in endpoints that underestimate the toxicity to birds.

Report: B.9.1.1.2/2
[redacted] 2014d
BAS 750 F-Avian dietary toxicity test in ducklings of the mallard duck (*Anas platyrhynchos*)
2014/1117035
Guidelines: EPA 850.2200, OECD 205, EPA 850.2000
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8% (tolerance ± 1%).

B. STUDY DESIGN

Test species: Mallard duck (*Anas platyrhynchos*) ducklings age 5 days, hatched from eggs of animals visually indistinguishable from wild birds. Source: Deindl GmbH & Co. KG Geflügelzucht und –vertrieb, Rietberg, Germany.

Test design: The test substance was administered via the diet for 5 days to mallard ducklings, with a post-exposure period of 3 days; 10 unsexed birds per test concentration and control group were used with random allocation based on weight; assessment for mortality and clinical signs was carried out four times on the first day of exposure and daily thereafter.

Housing: Steel wire pens (1.3×0.65×1.3m) with wire mesh floors covered with a plastic mat, a food hopper, toys and containers with drinking water and hay. Area of 0.845m^2 for 10 test organisms.

Organism examinations: Birds were weighed individually on days 0, 5 and 8. The mean body weight was calculated for each of these days for each pen. Mean food consumption per bird and day for the substance feeding and the post-exposure period was calculated from the daily mean food consumption of each group. Gross post-mortem examination was conducted for all birds that died during the test period and all birds sacrificed by CO_2 asphyxia at the end of the post-exposure period.

Endpoints: Mortality, clinical signs, feed consumption, body weight (b.w.) and gross-pathological examinations. Calculation of LD_{50}, LC_{50} and NOEL.

Test concentrations: 0 (Control), 1480, 2222, 3333, 5000 and 7500 mg a.s./kg diet.

Test conditions: Temperature: 17.1-21.6°C (21±2°C limits with time below limits of 1d 7h and 30 minutes)

Relative humidity: 49.6-100% (45%-70% limits with time above limit of 7 d, 7h and 15 minutes)

Photoperiod:	14 hours light: 10 hours dark, light intensity: 60-95lux in the middle of the cages on the floor level, warm light fluorescent lamps.
Analytics:	The test substance concentrations were analysed using HPLC.
Statistics:	LC_{50} and LDD_{50} calculation (mortality): Probit-analysis according to Finney and Goodness-of-Fit-Test (Pearson Chi-Square); NOEL calculation: Fisher's exact test (one-sided); comparison of b.w.: Dunnett test (two-sided). SAS System was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (EPA 850.2200(2012)):

- Random bird assignment
- ≤10% control mortality or moribund birds (0%)
- Maintenance of concentrations within ±20% of the nominal (92.9-110%)
- 5 day test substance administration (5d)
- ≥10 birds for each test concentration (10)
- Test substance administered within the diet
- ≥5 test substance concentrations and controls (5 and a control)

The results of the analytical verification of the test substance concentration in the diet were within a range of 92.9% to 110% of the nominal concentrations during the test. The biological results are therefore based on the nominal values.

No mortality was observed in the control group. The highest concentration tested causing no substance-related mortality was 3333 mg active substance/kg diet, corresponding to a daily dose of 506 mg a.s./kg b.w. Substance-related mortality was observed in the test concentrations ≥ 5000 mg active substance/kg diet, most likely caused by starvation. No other toxic signs were observed at the highest test concentration. The ducklings that died had a markedly reduced body weight. The calculated LC_{50} was 8347 mg active substance/kg diet (95% confidence interval: 6068 – 64610 mg active substance/kg diet; extrapolated value), exceeding the highest tested concentration group of 7500 mg active substance/kg diet. The LDD_{50} calculated on the basis of daily doses was 1213 mg active substance/kg bodyweight/day. No clinical signs of toxicity, related to the test substance, were observed in any treatment group. During the 5-day exposure period, the food consumption was decreased markedly in all tested concentration groups to 18.5-55.1% of the food consumption of the control group. The decrease followed generally a concentration-related trend, with some inconsistency. The food consumption was low enough to expect mortality from starvation in all tested groups. Mortality was observed, however, only in the two highest test concentrations.

During the 3-day post-exposure period, the food consumption of the surviving ducklings in all concentration groups returned to values between 64 and 104 % of the food consumption in the control group without a clear concentration-related trend. No excessive spill of food was observed for any of the test groups over the whole observation period. The body weights of the surviving ducklings on days 5 and 8 and the body weight development were statistically significantly decreased in all groups that were exposed to the test substance. The decrease followed a concentration-related trend. In the two highest concentrations, the body weight development was negative during the exposure period. In many birds of the treatment groups that were sacrificed on day 8, a discoloration of the liver was detected during the gross post mortem examination. The histological examination revealed a multifocal coagulation necrosis and fatty degeneration. The finding was often seen in combination with reduced body size and is most likely a consequence of starvation. No abnormalities were observed in the control group. Results are presented in the table below.

Table B.9.1.1.2-2: Avian dietary toxicity of BAS 750 F to the mallard duck (*Anas platyrhynchos*)

Parameter	Group [mg a.s./kg diet]					
	Control	**1480**	**2222**	**3333**	**5000**	**7500**
Daily dose [mg a.s./kg b.w.] 1)	not applicable	244	475	506	446	763
Mortality [%] (n=10)	0.0	0.0	0.0	0.0	40.0	30.0
Mean feed consumption during days 1 to 5 [g feed/bird/day]	31	13.6	17.1	11.2	5.7	6.6
Mean body weight on day 0 and day 5 [g/bird]	79.4/165.3	64*/94.4**	78.5/94.9**	77.6/81.2**	71.1/64.9**	73.7/60.3**
Clinical signs	None	None	None	None	None	None
	Endpoints [mg a.s./kg diet]					
LC_{50}	8347					
NOEL	<1480					
	Endpoint [mg a.s./kg b.w./day]					
LDD_{50}	1213					
NOEL	<244					

a.s.=active substance
b.w.=body weight
Statistic Profile=Dunnett test (two-sided), * p<=0.05, ** p <=0.01,

1) Based on measured concentration of active substance in the diet
2) The summary body weights are based on the mean body weights per duckling.

III. CONCLUSION

Under the conditions of this study, the LC_{50} for mallard ducklings (*Anas platyrhynchos)* was 8347 mg a.s./kg diet (extrapolated value). The LC_{50} exceeded the highest tested dietary concentration group of 7500 mg a.s./ kg diet. Calculated on the basis of daily doses, the LDD_{50} was 1213 mg a.s./kg b.w./day. The "No Observed Effect Level" (NOEL) was <1480mg a.s./kg diet. The NOEL calculated on the basis of daily dose was <244 mg a.s./kg b.w./day.

RMS Comment: The RMS notes that food avoidance and the toxic effect of the item has impacted food consumption and bodyweight data. Consequently, higher concentration dose groups resulted in reduced daily doses. Therefore, despite some clear effects on mortality, the effects cannot be separated from the issue of food avoidance meaning doses cannot reliably be attributed to effects of the active substance. Additionally, the effect of food avoidance may be causing starvation and contributing to the mortalities. These issues will result in endpoints that underestimate the toxicity to birds. The study has proposed an extrapolated value for the LC_{50}, however as there is no clear dose-response relationship and the confidence limits for the value range over a factor of 10, the proposed value cannot be accepted.

The RMS agrees that the temperature gradient should be between 22-38°C, although the minimum temperature reached 17.1°C. Given the temperature gradient with the ceramic heater and no negative effects observed in the controls, the test conditions exceeding the recommended values are not expected to have adversely affected the test. Additionally the mean body weight for the 1480 mg a.s./kg diet group had significantly reduced mean body weight at day 0, which would make any

significant decline in body weight for this group over the course of the test more difficult to define from the initial reduced weight.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Report: B.9.1.1.3/1
[redacted] 2014a
BAS 750 F: A reproduction study with the northern bobwhite
2013/1281276
Guidelines: EPA 850.2300, OECD 206
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, Batch No.: COD-001740, purity: 98.8%

B. STUDY DESIGN

Test species: Northern bobwhite quails (*Colinus virginianus*), phenotypically indistinguishable from wild type. The adults were 23 weeks of age at the initiation of the test (before beginning of first egg-laying period). Supplied by Trace Pheasantry, 288 Levengood Road, Douglassville, PA 19518.

Test design: Groups of 1 male and 1 female in a pen per replicate. 18 pens (25×51cm) were allocated to the control and each treatment group. All birds were given feed and water *ad libitum* during acclimation and testing. The study period was divided into five phases:

1. Acclimation to laboratory conditions – 6 weeks;
2. Pre-photostimulation – 8 weeks;
3. Pre-egg laying (with photostimulation) – 3 weeks;
4. Egg laying period – 10 weeks;
5. Post-adult termination (final incubation, hatching and 14-day offspring rearing period) – 6 weeks.

Eggs were collected daily from all pens and stored in a cold room. At the end of a weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for egg shell thickness measurement.

Cracked or abnormal eggs were recorded and discarded. All eggs not discarded or used for egg shell thickness measurements were placed in an incubator. On day 21 of incubation, eggs were moved to a hatcher. Young birds were maintained for 14 days. Adult birds were sacrificed after the egg-laying period, young birds after 14 days.

Endpoints: Adult birds: mortalities, clinical observations, gross necropsy, adult body weight and adult feed consumption

Reproductive parameters: Eggs laid/hen/day, eggs cracked of eggs laid, fertile eggs of eggs set, viable embryos of eggs set, live 3-week embryos of viable embryos, hatchlings of 3 week-Embryos, body weight hatchlings, 14-day old survivors of hatchlings, hatchlings of eggs set, hatchlings of

fertile eggs, hatchlings/pen/day, 14-day old survivors/pen/day, 14-day old survivors of eggs set, offspring body weight and egg shell thickness

Test concentrations: 0 (Control), 150, 285 and 543 mg a.s./kg diet BAS 750 F (nominal).

Test conditions: Temperature and relative humidity refer to mean conditions,
Adult bobwhite study room:
Temperature: 19.3 ± 1.6°C (SD);
Relative humidity: 38 ± 14% (SD);
Ventilation: 15 times the room air/h;
Photoperiod: 7 hours light (week 1 – 8) approximately 331lux, lengthened photoperiod to 17 hours light (week 9 to the end of study) approximately 344lux and during egg-laying approximately 242lux.

Egg collection and storage:
Collected daily, stored in cold room
Temperature: 13.7 ± 0.3°C (SD)
Relative humidity: 67 ± 8% (SD).

Eggs set for incubation
Temperature: 37.5 ± 0.0°C (SD)
Relative humidity 55 ± 0.0°C (SD)

The eggs were transferred to the hatcher on day 21
Temperature: 37.3 ± 0.0°C (SD)
Relative humidity: approximately 57 ± 1% (SD)

Hatchlings:
Brooding compartment
Temperature: approximately 38°C from hatching until the birds were 14 days of age;

Room:
Temperature: 26.5 ± 2.7°C (SD)
Relative humidity: 20 ± 8%
Photoperiod: 16 hours light per day

Analytics: The test substance concentrations were analysed using HPLC with UV detection. The limit of quantitation was 10.0ppm a.s.

Statistics: Analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Comparison of control and treatment groups: Dunnett´s multiple comparison procedure. Percentage data: Dunnett´s method following arcsine square root transformation

II. RESULTS AND DISCUSSION

Validity criteria:
All the validity criteria were met (EPA 850.2300 (2012)):

- Birds were randomly assigned to treatment and control pens.
- ≤10% of the control birds died or became moribund during the test (0%).
- ≥29 average number of eggs laid per control hen (45)
- ≥80% of control eggs set had viable embryos (88%)
- ≥97% of live control embryos are survivors at 3-weeks (99%)

- ≥85% of viable control embryos became normal hatchlings (89%)
- ≥71% of control eggs set became normal hatchlings (80%)
- ≥77% of normal control hatchlings became 14-day survivors (83%)
- ≥0.20mm average control eggshell thickness (0.229mm)
- ≤13% of control eggs cracked (3%)

Analytical measurements:
The result of the analytical verification of the test substance was 97%. Concentrations of the test substance in the diet were adjusted to 100% active substance based on test substance purity of 98.8%. Mean concentrations and standard deviations for the three test concentrations were 156 ± 4.18, 293 ± 4.59, and 569 ± 7.66 mg a.s./kg diet for the nominally 150, 285, and 543 mg a.s./kg diet concentrations, respectively. Additionally, recovery was 88-93% on week 1 after 7 days, 98-103% on week 12 after 7 days and 97-103% of week 20 after 7 days.

Biological results:
No adult mortalities occurred in the control group or the 285 mg a.s./kg diet treatment group, although one incidental mortality occurred in each of the 150 and 543 mg a.s./kg diet treatment groups. Due to the nature of the lesions observed, the mortalities that occurred were considered to be unrelated to treatment.

No overt signs of toxicity were observed at any of the concentrations tested. Clinical signs observed included a ruffled appearance, a thin appearance and slight loss of coordination. However, these signs were limited to few birds and lasted for one day only. Other observations included incidental injuries associated with pen wear such as foot lesions, feather loss, rump lesions, lameness and a slightly irritated eye. Otherwise, all birds have been normal in appearance and behaviour.

There were no apparent treatment-related effects upon adult body weight at any of the concentrations tested. There were no statistically significant differences in body weight between the control group and the 150, 285, and 543 mg a.s./kg diet treatment groups at any of the body weight intervals. Summarised results see Table B.9.1.1.3/1-1.

There were no apparent treatment-related effects upon feed consumption at the 150 or 285 ppm a.s. test concentration. However, at the 543 mg a.s./kg diet test concentration there was a slight reduction in feed consumption during week 1 that may have been related to treatment.

No statistically significant differences between the control group and the 150 mg a.s./kg diet treatment group were observed at any of the feed consumption intervals. In the 285 mg a.s./kg diet treatment group, there were slight, but statistically significant ($p<0.05$) increases in feed consumption during week 19 and 20. These differences were not considered treatment related since they were neither consistent over time nor concentration responsive. At the 543 mg a.s./kg diet test concentration, there was a slight reduction in mean feed consumption during week 1 that was statistically significant ($p<0.05$). This slight reduction may have been due to avoidance but lasted only one week.

Reproductive results:
There were no apparent treatment related effects upon egg shell thickness at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters at 150 and 285 mg a.s./kg diet. At the 543 ppm a.s. test concentration, there was a statistically significant ($p\leq0.01$) reduction in eggs laid per hen per day. While not statistically significant, the reduction in eggs laid per hen per day was also reflected in reductions in the number of hatchlings and 14-day old survivors per hen per day. Additionally, while not statistically significant, at the 543 mg a.s./kg diet test concentration there was a reduction in offspring survival.

There were no apparent treatment related effects upon offspring body weight at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in the body weight of hatchlings from any of the treatment groups and no statistically significant difference in the body weight of 14-day old survivors at the 150 and 543 mg a.s./kg diet test concentrations. However, when compared to the control group, there was a slight increase in 14-day old survivor body weights at the 285 mg a.s./kg diet treatment group that was statistically significant ($p \leq 0.01$). Since the difference was slight, represented an improvement in performance and was not concentration responsive, it was not considered to be treatment related. Results are summarised in Table B.9.1.1.3/1-2 and Table B.9.1.1.3/1-3.

Table B.9.1.1.3/1-1: Effects of BAS 750 F on the parental generation of the northern bobwhite quail (*Colinus virginianus*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	**150**	**285**	**543**
No. of replicates (1 male and 1 female per replicate/pen)	18	18	18	18
No. of substance-related mortalities of adult birds	0	0	0	0
Adult body weight [g] at the end of study (male/female)	223/244	219/242	224/241	219/244
Gain of adult body weight [g] at the end of study (male/female)[1)]	18/42	16/38	18/41	16/40

Table B.9.1.1.3/1-2: Effects of BAS 750 F on the reproduction of the northern bobwhite quail (*Colinus virginianus*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	**150**	**285**	**543**
Number of surviving replicates	18	17	18	17
Total eggs laid	815	729	739	473
Eggs laid/hen	45	43	41	28
Eggs laid/hen/day	0.49	0.47	0.45	0.30**
Eggs cracked	22	12	27	4
Mean egg shell thickness (mm)	0.229 ± 0.014	0.239 ± 0.016	0.233 ± 0.014	0.231 ± 0.015
Eggs set	691	639	628	390
Viable Embryos	613	614	548	360
Mean body weight (g) of hatchlings per group	5.9 ± 0.7	6.0 ± 0.4	6.1 ± 0.4	5.5 ± 0.6
Live 3-week embryos	605	611	541	350
Mean bodyweight (g) of 14-day old survivors	26 ± 3	26 ± 3	29** ± 2	24 ± 3
Hatchlings	553	582	500	335
Hatchlings/hen	31	34	28	20
14-day old survivors	501	530	450	256
14-day old survivors/hen	28	31	25	15

** Significantly different from the control at $p < 0.01$ (Dunnett´s t-test).

Table B.9.1.1.3/1-3: Effects of BAS 750 F on the reproduction of the northern bobwhite quail (*Colinus virginianus*) expressed as percentages

Parameter	Treatment group [mg a.s./kg diet]			
	Control	150	285	543
% viable embryos/eggs set	88	96	87	92
% live 3-week embryos/viable embryos	99	100	99	97
% hatchlings/live 3-week old embryos	89	96	93	95
% hatchlings/eggs set	80	92	80	84
% 14-day old survivors/eggs set	71	82	72	64
% 14-day survivors of hatchlings	83	89	91	75
% cracked eggs of eggs laid	3	2	3	1

III. CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight at the 150, 285 or 543 mg a.s./kg diet test concentrations. There were no treatment-related effects upon feed consumption at the 150 or 285 mg a.s./kg diet test concentrations. At the 543 mg a.s./kg diet test concentration, there was a slight reduction in feed consumption during Week 1 that may have been related to treatment. There were no treatment related effects upon any of the reproductive parameters measured at the 150 and 285 mg a.s./kg diet test concentration. However, at the 543 mg a.s./kg diet test concentration, there was a marked, statistically significant ($p \leq 0.01$) reduction in egg production and a reduction in offspring survival (not statistically significant). The no-observed-effect concentration (NOEC) for northern bobwhite quails exposed to BAS 750 F in the diet during the study was 285 mg a.s./kg diet (25.3 mg a.s./kg b.w./day).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are a NOEC of 285 mg a.s./kg diet (25.3 mg a.s./kg b.w./day). The relative humidity of the adult study room and the brooding pens were below the recommended levels of 45-70%. Ideally the water should be changed every day rather than every 2-3 days as it is in a trough. Given that all the validity criteria were met and no other negative effects were observed in the controls, these deviations are not thought to have adversely affected the experiment. Although there is an apparent dose response in the number of eggs laid/ hen/ day, this does not follow through to other reproductive parameters such as hatchlings/hen. Therefore no changes to the NOEC are proposed. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
NOEC of 285 mg a.s./kg diet (25.3 mg a.s./kg b.w./day)

Report: B.9.1.1.3/2
[redacted] 2015a
BAS 750 F: A reproduction study with the mallard
2015/7005819
Guidelines: EPA 850.2300, OECD 206
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance:	BAS 750 F, Batch No.: COD-001740, purity: 98.8% (tolerance ± 1.0%)

B. STUDY DESIGN

Test species:	Mallard (*Anas platyrhynchos)*, phenotypically indistinguishable from wild type; adults, age: 26 weeks of age at the initiation of the test (before beginning of first egg-laying period); supplier: Whistling Wings 113 Washington Street, P.O. Box 1A, Hanover, IL 61041.
Test design:	Mallards approaching their first breeding season were kept in a group of 1 male and 1 female in a pen per replicate. 18 pens (75×90cm) were allocated to the control and each treatment group. All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. The study period was divided into five phases: 1. Acclimation – 6 weeks; 2. Pre-photostimulation – 10 weeks; 3. Pre-egg laying (with photostimulation) – 0 weeks. Egg production was considered to have begun at photostimulation; 4. Egg laying period – 10 weeks; 5. Post-adult termination (final incubation, hatching and 14-day offspring rearing period – 6 weeks. Eggs were collected daily from all pens and stored in a cold room. At the end of a weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for egg shell thickness measurement. Cracked or abnormal eggs were recorded and discarded. All eggs not discarded or used for egg shell thickness measurements were placed in a NatureForm Incubator (Model No. NMC 4000) after washing. On day 24 of incubation, eggs were transferred to a hatcher. Young birds were maintained for 14 days. Adult birds were sacrificed after the egg-laying period, young birds after 14 days.
Endpoints:	Adult birds: mortalities, clinical observations, gross necropsy, adult body weight and adult feed consumption Reproductive parameters: Eggs laid/hen/day, eggs cracked of eggs laid, fertile eggs of eggs set, viable embryos of eggs incubated, live 3-week embryos of viable embryos, hatchlings of 3 week-embryos, 14-day old survivors of hatchlings, hatchlings of eggs incubated, hatchlings of fertile eggs, hatchlings/pen/day, 14-day old survivors/pen/day, 14-day old survivors of eggs incubated, hatchling´s body weight and egg shell thickness.
Test concentrations:	0 (Control), 150, 300 and 600 mg a.s./kg diet BAS 750 F in the diet (nominal).
Test conditions:	Temperature and relative humidity refer to mean conditions Adult mallard study room: Temperature: 21.3 ± 1.6°C (SD); Relative humidity: 51 ± 15% (SD); Ventilation: 15 times the room air/h;

Photoperiod: 8 hours light or less per day (week 1 – 10) approximately 226lux, photoperiod of 17 hours light (week 11 to the end of study) approximately 234lux and during egg-laying approximately 248lux (fluorescent lights).

Egg collection and storage:
Collected daily, stored in cold room
Temperature: 12.3 ± 1.2°C (SD),
Relative humidity: 84 ± 4% (SD).

Eggs set for incubation:
Temperature: 37.4 ± 0.0°C (SD),
Relative humidity: approximately 55 ± 0.0°C (SD);

Hatcher on day 24:
Temperature: 37.3 ± 0.0°C (SD),
Relative humidity: approximately 60 ± 1% (SD)

Hatchlings:
Brooding compartment temperature approximately 38°C from hatching to the birds were 6 days of age; average ambient room temperature 25.5 ± 0.0°C (SD), relative humidity: 74% ± 8%; photoperiod: 16 hours light per day

Analytics: The test substance concentrations were analysed using HPLC with UV detection. The limit of quantitation was 10.0ppm a.s.

Statistics: Analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Comparison of control and treatment groups: Dunnett´s multiple comparison procedure. Percentage data: Dunnett´s method following arcsine square root transformation

II. RESULTS AND DISCUSSION

All the validity criteria were met:

1. Birds were randomly assigned to treatment and control pens.
2. ≤10% of the control birds died or became moribund during the test (0%).
3. ≥29 average number of eggs laid per control hen (43)
4. ≥80% of control eggs set had viable embryos (83%)
5. ≥94% of live control embryos are survivors at 3-weeks (99%)
6. ≥52% of viable control embryos became normal hatchlings (89%)
7. ≥44% of control eggs set became normal hatchlings (73%)
8. ≥94% of normal control hatchlings became 14-day survivors (99%)
9. ≥0.316mm average control eggshell thickness (0.391mm)
10. ≤13% of control eggs cracked (0%)

Analytical measurements:
The result of the analytical verification of the test substance was 101-104% for samples taken during the test, 92-101% after 7 days in the feeders on week 1, 92-100% after 7 days in the feeders on week 12 and 89-97% after 7 days in the feeders on week 20. Concentrations of the test substance in the diet were adjusted to 100% active substance based on test substance purity of 98.8%. Mean concentrations and standard deviations for the three test concentrations were 158 ± 6.06, 314 ± 8.37, and 615 ± 22.0 mg a.s./kg diet for the nominally 150, 300, and 600 mg a.s./kg diet concentrations, respectively.

Biological results:
A single incidental mortality occurred in this study. The female from Pen 1361 of the 600 ppm a.s. treatment group was found dead on Day 2 of Week 17. Prior to being found dead the female was noted as normal in appearance and behaviour. At necropsy the hen weighed 863 grams, her spleen and pancreas were pale, kidneys slightly pale, and there were extensive lesions of egg yolk peritonitis in the abdominal cavity. The hen's ovary was regressing. Necropsy of the female's penmate was unremarkable.

At the Week 2 body weight measurements, the plumage on the nominal hen from Pen 1335 of the 150 mg a.s./kg diet treatment group was cryptic but indicated that the bird was a male. The pair was euthanized and data from this pen were excluded from the test. Necropsy confirmed the sex of the nominal female as male. Otherwise, necropsy of both birds was unremarkable. No other mortalities occurred during the course of the study. Due to the nature of the lesions observed, the mortalities that occurred were considered to be unrelated to treatment.

No overt signs of toxicity were observed at any of the test concentrations. Observations included incidental injuries associated with pen wear and included foot lesions, feather loss, and an unkempt or thin appearance. Except for incidental findings, all birds appeared normal throughout the study.

All surviving adults were subjected to gross necropsy following adult termination. Findings from birds in the 150, 300, and 600 mg a.s./kg diet test concentrations were considered to be incidental to treatment.

There were no apparent treatment-related effects upon adult body weight at any of the concentrations tested. There were no statistically significant differences in body weight between the control group and the 150 mg a.s./kg diet treatment groups at any of the body weight intervals. At the 300 mg a.s./kg diet test concentration, the mean body weight of hens at Week 8 of the test was slightly higher than the control group ($p<0.05$). However, the increase was slight and represented an improvement and therefore it was considered to be unrelated to treatment. At the 600 mg a.s./kg diet test concentration, there was a slight reduction in weight gain between Week 8 and adult term, and overall, and mean weight of hens at adult term that were statistically significant ($p<0.05$). However, the differences were slight and primarily due to the hen in Pen 1365 that was noted as thin and weighing 729 grams at adult termination. For summarised results, see Table B.9.1.1.3/2-1.

There were no apparent treatment-related effects upon feed consumption at the 150, 300, or 600 mg a.s./kg diet test concentrations and no statistically significant differences between the control group and the 600 mg a.s./kg diet treatment group were observed at any of the feed consumption intervals. At the 150 test concentration there was a slight increase in mean feed consumption during Week 5 of the test that was statistically significant ($p<0.05$). At the 300 mg a.s./kg diet test concentration there were slight increases in mean feed consumption that were statistically significant at $p<0.05$ during Weeks 2, 3, and 6, and statistically significant at $p <0.01$ during Weeks 1 and 5 of the test. The increases in mean feed consumption were slight, not concentration responsive, and appeared to have been related to feed wastage by individual pens and were therefore not considered to be biologically meaningful.

There were no treatment-related effects upon reproductive performance measured at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters measured in the 150, 300, or 600 mg a.s./kg diet treatment groups.

There were no apparent treatment-related effects upon offspring body weights at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in weights of hatchlings for any of the treatment groups or for 14-day old survivors at the 300 and 600 mg a.s./kg diet test concentrations. At the 150 mg a.s./kg diet test concentration, there

was a slight but statistically significant ($p<0.05$) increase in weights of 14-day old survivors. However, the increase in 14-day old body weights was slight, not concentration responsive, and represented an improvement, and therefore was not considered to have been related to treatment. The decrease in body weight was primarily due to two female birds with body weights of 746 and 729g rather than the average of 1063g for females when these birds are removed from the 600 mg a.s./kg bw/d. Equally when these two females are removed, average body weight gain increases from 75mg/L to 111mg/L. These effects were limited to females only, and no dose response relationship appeared to be apparent with the other concentrations. Results are summarised in Table B.9.1.1.3/2-2 and Table B.9.1.1.3/2-3.

Table B.9.1.1.3/2-1: Effects of BAS 750 F on the parental generation of the mallard (*Anas platyrhynchos*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	150	300	600
No. of replicates (1 male and 1 female per replicate/pen)	18	18	18	18
No. of substance-related mortalities of adult birds	0	0	0	0
Adult body weight [g] at the end of study (male/female)	1090/1127	1100/1138	1090/1149	1088/1025*
Adult body weight at the end of study as a percentage of the control (male/female)	-	101/101%	100/102%	100/91%
Range (standard deviation in brackets) of adult body weight at the end of study (male/female)	979-1177 (86)/ 906-1391 (124)	903-1230 (88)/ 938-1306 (103)	987-1220 (59)/ 947-1438 (117)	982-1235 (76)/ 729-1190 (135)
Gain of adult body weight [g] at the end of study (male/female)	22/168	37/174	21/185	18/75*
Gain of adult body weight at the end of study as a percentage of the control (male/female)	-	168/104%	95/110%	81/45%
Range (standard deviation in brackets) of gain of adult body weight at end of study (male/female)	-80-135 (57)/ 37-492 (111)	-55-171 (56)/ 4-299 (85)	-131-136 (67)/ 46-316 (79)	-150-136 (81)/ -210-237 (129)

* Significantly different from control at $p \leq 0.05$ (Dunnett's t-test)

Table B.9.1.1.3/2-2: Effects of BAS 750 F on the reproduction of the mallard (*Anas platyrhynchos*)

Parameter	Treatment group [mg a.s./kg diet]			
	Control	150	300	600
Number of surviving replicates	18	17	18	17
Total eggs laid	779	728	813	684
Eggs laid/hen[1)]	43	43	45	40
Eggs laid/hen/day[2)]	0.62	0.61	0.65	0.57
Eggs cracked	0	3	3	1
Mean egg shell thickness (mm)	0.391 ± 0.022	0.395 ± 0.020	0.380 ± 0.031	0.383 ± 0.027
Eggs set	698	644	716	614
Viable Embryos	559	602	686	577
Mean body weight (g) of hatchlings per group	36 ± 2.0	35 ± 1.9	35 ± 3.9	36 ± 3.3
Live 3-week embryos	555	593	683	569
Mean bodyweight (g) of 14-day old survivors	288 ± 17	308* ± 22	297 ± 15	289 ± 26
Hatchlings	492	520	645	526
14-day old survivors	488	513	643	522
14-day old survivors/pen	27	30	36	31

[1] The total number of eggs laid in each group.
[2] Based on 70 days of egg production.
* Significantly different from control at $p \leq 0.05$ (Dunnett's t-test).

Table B.9.1.1.3/2-3: Effects of BAS 750 F on the reproduction of the mallard (*Anas platyrhynchos*) expressed as percentages

Parameter	Treatment group [mg a.s./kg diet]			
	Control	150	300	600
% viable embryos/eggs set	83	94	90	94
% live 3-week embryos/viable embryos	99	99	100	99
% hatchlings/live 3-week old embryos	89	88	94	92
% hatchlings/eggs set	73	81	84	87
% 14-day old survivors/eggs set	72	81	84	86
% 14-day survivors of hatchlings	99	99	100	99
% cracked eggs of eggs laid	0	0	1	0

Percent values represent replicate means for each experimental group. Differences between control group and each of the treatment groups were not significant (p >0.05, Dunnett's t-test).

III. CONCLUSION

There were no treatment-related mortalities, overt signs of toxicity, or treatment related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at the 150, 300 or 600 mg a.s./kg diet test concentrations. The no-observed-effect concentration for mallard exposed to BAS 750 F in the diet during the study was therefore considered to be 600 mg a.s./kg diet (80.5 mg a.s./kg b.w./day), the highest concentration tested.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are a NOEC of 600 mg a.s./kg diet (80.5 mg a.s./kg b.w./day). Although statistically significant in reduction in body mass and gain of adult body weight was observed at 600ppm, this was not considered test substance related as is due to the reduction in the body weight of two female birds. Similar body reductions were not observed in the 15 surviving female birds at this concentration and in any of the surviving male birds, and no dose response relationship was present for the other concentration. Furthermore the range and standard deviations for these data (Table B.9.1.1.3/2-3) indicate both that the recorded weights were highly variable, and that these two birds are considered to be outliers. Therefore the significant reduction is not considered to be sufficient for reducing the NOEC to 300 mg a.s./kg diet. The environmental conditions in several areas exceeded the recommended levels. The light intensity of the adult pens should be around 65lux. Eggs should be stored in a cold room of 13-16°C with a relative humidity of 55-80%, and the relative humidity of the incubator should be around 70%. Given that no negative effects were observed in the control, these deviations are not expected to have had an adverse effect on the study. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
NOEC of 600 mg a.s./kg diet (80.5 mg a.s./kg b.w./day)

B.9.1.2. Effects on terrestrial vertebrates other than birds

The endpoints listed in Table B.9.1.2-1 below have been included as part of the toxicology dossier, but are applicable mammalian endpoints for ecotoxicology assessments. For the critical endpoint, $NOAEL_{parents,}$ at 75mg/kg bw/d increased cholesterol in males, liver weight in males and females, and increased alkaline phosphatase concentrations in males and females was reported.

Table B.9.1.2-1 Summary of endpoints from the mammalian toxicity section

Organism	Test substance	Timescale/ Test type	Endpoint	Toxicity value	Reference
Rat	BAS 750 F	Oral, 1 d Acute	LD_{50}	> 2000 mg a.s./kg bw	[redacted] 2013c (CA 5.2.1/1)
Rat	BAS 750 01 F	Oral, 1 d Acute	LD_{50}	> 2000 mg formulation/kg bw	[redacted] 2015a (CP 7.1.1/1)
Rat	BAS 750 F	Dietary Reproductive toxicity Two-generation study	$NOAEL_{repreoduction}$ $NOAEL_{parents}$ $NOAEL_{offspring}$	200 mg a.s./kg bw/d 25 mg a.s./kg bw/d 75 mg a.s./kg bw/d	[redacted] 2015c (CA 5.6.1/1)
Rat	BAS 750 F	Oral Developmental toxicity	$NOAEL_{maternal}$ $NOAEL_{developmental}$	150 mg a.s./kg bw/d 400 mg a.s./kg bw/d	[redacted] 2015a (CA 5.6.2/1)
Rabbit	BAS 750 F	Oral Developmental toxicity	$NOAEL_{maternal}$ $NOAEL_{developmental}$	25 mg a.s./kg bw/d 25 mg a.s./kg bw/d	[redacted] 2015b (CA 5.6.2/2)

B.9.1.2.1. Acute oral toxicity to mammals

No studies have been submitted as part of the ecotoxicology dossier, but mammalian studies have been submitted as part of 3CA B.6.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

No studies have been submitted as part of the ecotoxicology dossier, but mammalian studies have been submitted as part of 3CA B.6.

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

No studies have been submitted. However, a bioaccumulation study using fish has been conducted and is summarised in section B.9.2.2/3. Further consideration of bioconcentration has been included in the secondary poisoning risk assessment for birds and mammals in the relevant PPP dossiers

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

No additional studies on other terrestrial vertebrates are required.

B.9.1.5. Potential for endocrine disruption

Member States should note that there are currently no defined criteria for identifying endocrine disruptors or interpreting the significance of any affects in ecotoxicology studies under the Commission Regulation (EU) No. 2009/1107. As a result of this, endpoints have not been defined and a risk assessment has not been conducted. Discussion of endocrine effects has been undertaken within the toxicology section (III CA B.6.8.3), although from an ecotoxicological perspective it cannot be concluded if endocrine disruptive effects are or are not taking place.

B.9.2. EFFECT ON AQUATIC ORGANISMS

Table B.9.2-1: List of studies and endpoints for aquatic organisms exposed to the active substance and metabolites

Organism	Substance	Endpoint	Value [mg a.s./L] (except BCF & sediment endpoint of spiked sediment study)	Reference
Acute toxicity to fish				
Oncorhynchus mykiss	BAS 750 F	96 h LC_{50}	**0.532**	B.9.4.1.1/1, [redacted], 2014a
Cyprinus carpio		96 h LC_{50}	1.126	B.9.4.1.1/2, [redacted] 2015c
Danio rerio, (Syn. *Brachydanio rerio*)		96 h LC_{50}	0.906	B.9.4.1.1/3, [redacted] 2015a
Cyprinodon variegatus		96 h LC_{50}	0.761	B.9.2.1.1/4, [redacted] 2014a
Chronic toxicity to fish				
Cyprinodon variegatus (ELS)	BAS 750 F	35 d NOEC	0.147	B.9.2.2/1, [redacted] 2015a
Danio rerio (ELS)		36 d NOEC	0.027	B.9.2.2/2, [redacted]

Organism	**Substance**	**Endpoint**	**Value [mg a.s./L]** (except BCF & sediment endpoint of spiked sediment study)	**Reference**
				2015b
Endocrine disruption to fish				
Danio rerio (FSDT)	BAS 750 F	69 d NOEC	≥ 0.045	B.9.2.3/1, [redacted] 2015a
Acute toxicity to aquatic invertebrates				
Daphnia magna	BAS 750 F	48 h EC_{50}	**0.944**	III CA B.9.2.4.1/1, Brzozowska., 2014a
Americamysis bahia #		48 h LC_{50}[2] 96 h LC_{50}	1.53 1.30	B.9.2.4.1/2, VanHooser, 2014a
Crassostrea virginica		96 h EC_{50}	0.947	B.9.2.4.1/3, VanHooser, 2014b
Chronic toxicity to aquatic invertebrates				
Daphnia magna	BAS 750 F	21d NOEC 21 d EC_{10}	0.0091 0.0161	B.9.2.5/1, Janson, 2014a
Daphnia longispina		21d NOEC 21 d EC_{10}	0.0342 0.0564	B.9.2.5/2, Janson, 2014b
Daphnia pulex		21d NOEC 21 d EC_{10}	0.0276 0.0567	B.9.2.5/3, Janson, 2015a
Americamysis bahia #		28d NOEC	≥ 0.0132	B.9.2.5/4, Dinehart, 2016a
Acute/ sub-chronic toxicity to sediment dwelling aquatic invertebrates				
Chironomus dilutes (spiked sediment)	BAS 750 F	10d NOEC	7.08 mg /kg dry sediment	B.9.2.6/1, Clark, 2015a
		10d EC_{50}	> 96 mg /kg dry sediment	
Hyalella azteca (spiked sediment)		10d NOEC	≥ 100 mg /kg dry sediment	B.9.2.6/4, Clark, 2015b
		10d EC_{50}	> 100 mg /kg dry sediment	
Leptocheirus plumulosus (spiked sediment)		10d NOEC	≥ 95 mg /kg dry sediment	B.9.2.6/5, Clark, 2015c
		10d LC_{50}	> 95 mg /kg dry sediment	
Chronic toxicity to sediment dwelling aquatic invertebrates				

Organism	**Substance**	**Endpoint**	**Value [mg a.s./L]** (except BCF & sediment endpoint of spiked sediment study)	**Reference**
Chironomus riparius (spiked sediment)	BAS 750 F	28 d NOEC	≥ 1.158 mg/kg dry sediment	B.9.2.6/3, Backfisch &Weltje, 2015b
Algae [1)]				
Pseudokirchneriella subcapitata	BAS 750 F	72 h E_rC_{50}	1.352	B.9.2.7.1/1, Brzozowska, 2014b
		72 h NOE_rC 72 h E_rC_{10}	0.103 0.904	
		72 h E_yC_{50}	0.777	
		72 h NOE_yC 72 h E_yC_{10}	<0.103 0.215	
Skeletonema costatum [#]		72 h E_rC_{50}	**0.679**	B.9.2.7.1/2, Bergfield, 2015a
		72 h NOE_rC 72 h E_rC_{10}	0.0985 0.373	
		72 h E_yC_{50}	0.479	
		72 h NOE_yC 72 h E_yC_{10}	0.0985 0.257	
Navicula pelliculosa		72 h E_rC_{50}	1.347	B.9.2.7.1/3, Bergfield, 2015b
		72 h NOE_rC 72 h E_rC_{10}	0.303 0.478	
		72 h E_yC_{50}	0.671	
		72 h NOE_yC 72 h E_yC_{10}	0.303 0.351	
Anabaena flos-aquae		72 h E_rC_{50}	> 3.08	B.9.2.7.1/4, Bergfield, 2015c
		72 h NOE_rC 72 h E_rC_{10}	≥ 3.08 >3.08	
		72 h E_yC_{50}	> 3.08	
		72 h NOE_yC 72 h E_yC_{10}	≥ 3.08 >3.08	
Macrophytes [1)]				
Lemna gibba	BAS 750 F	7 d E_rC_{50}	**> 2.017**	B.9.2.8/1,

Organism	**Substance**	**Endpoint**	**Value [mg a.s./L]** (except BCF & sediment endpoint of spiked sediment study)	**Reference**
		7 d NOE_rC 7d E_rC_{10}	≥ 2.017 > 2.017	Swierkot, 2014a
		7 d E_yC_{50}	> 2.017	
		7d NOE_yC 7d E_rC_{10}	≥ 2.017 > 2.017	
Bioconcentration				
Oncorhynchus mykiss (BCF; 14d uptake, 7 d depuration)	BAS 750 F	BCF_{KLg} (whole fish)	385	B.9.2.2/3, [redacted] 2015c
Metabolites				
Fish				
Oncorhynchus mykiss	1,2,4-triazole (Reg. No. 87084; M750F001)	96 h LC_{50}	498	EFSA Scientific Report (2008) 138, 1-80
	1,2,4-triazole (Reg. No. 87084; M750F001)	28 d NOEC	3.2	EFSA Scientific Report (2008) 138, 1-80
QSAR Data	M750F003	96 h LC_{50}	> 100	B.9.12
QSAR Data	M750F005	96 h LC_{50}	11.3	B.9.12
Oncorhynchus mykiss	M750F006	96 h LC_{50}	6.2	B.9.4.1.2/2, [redacted] 2016
Oncorhynchus mykiss	M750F007	96 h LC_{50}	> 7.20	B.9.4.1.2/1, [redacted] 2015b
QSAR Data	M750F008	96 h LC_{50}	> 1.96	B.9.12
Aquatic invertebrates				
Daphnia magna	1,2,4-triazole (Reg. No. 87084; M750F001)	48 h EC_{50}	> 100	EFSA Scientific Report (2008) 138, 1-80
Daphnia magna	M750F003	48 h EC_{50}	> 100	B.9.4.4.2/5 Haerthe N., 2016,
Daphnia magna	M750F005	48 h EC_{50}	> 8.58	B.9.4.4.2/3, Rzodeczko, 2015d
Daphnia magna	M750F006	48 h EC_{50}	4.42	B.9.4.4.2/2, Rzodeczko, 2015c

Organism	**Substance**	**Endpoint**	**Value [mg a.s./L]** (except BCF & sediment endpoint of spiked sediment study)	**Reference**
Daphnia magna	M750F007	48 h EC_{50}	> 10	B.9.4.4.2/1, Backfisch & Härthe, 2015a
Daphnia magna	M750F008	48 h EC_{50}	> 8.07	B.9.4.4.2/4, Rzodeczko, 2015e
Algae				
Pseudokirchneriella subcapitata [1)]	1,2,4-triazole (Reg. No. 87084; M750F001)	72 h E_rC_{50}	22.5	EFSA Scientific Report (2008) 138, 1-80
Pseudokirchneriella subcapitata [1)]	M750F003	72 h E_rC_{50}	> 100	B.9.4.7.2/5 Backfisch K., 2016
Pseudokirchneriella subcapitata [1)]	M750F005	72 h E_rC_{50}	> 8.57	B.9.4.7.2/4, Rzodeczko, 2016b
Pseudokirchneriella subcapitata [1)]	M750F006	72 h E_rC_{50}	1.42	B.9.4.7.2/3, Rzodeczko, 2016a
Pseudokirchneriella subcapitata [1)]	M750F007	72 h E_rC_{50}	> 10	B.9.4.7.2/1, Backfisch, 2015a
Pseudokirchneriella subcapitata [1)]	M750F008	72 h E_rC_{50}	4.08	B.9.4.7.2/2, Brzozowska-Wojoczek, 2015a
Sediment-dwelling aquatic organisms				
Chironomus riparius (spiked sediment study)	M750F003	28 d NOEC	≥ 1.944 mg/kg dry sediment	B.9.4.6/2, Backfisch & Weltje, 2015a

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and macrophytes), only the relevant endpoint(s) is used in the (tier 1) risk assessment.

Abbreviations: ELS = early life stage; FSDT = fish sexual development test; BCF_{KLg} = growth corrected kinetic bioconcentration factor normalized to 5% lipid content;

[#] Estuarine/Marine species.

[1)] In accordance to the EFSA Aquatic Guidance Document (EFSA 2013), only the EC_{50} values determined for the more relevant endpoint 'growth rate' (E_rC_{50}) are considered for the risk assessment for aquatic primary producers if both "growth rate" and "yield / biomass" endpoints are available.

[2] The applicant has argued for a 48h endpoint to be comparable to that of the 48h *Daphnia* study. In the absence of clear guidance, both 96h and 48h endpoints have been presented

B.9.2.1. Acute toxicity to fish

B.9.2.1.1 Acute toxicity to fish from the active substance

Report:	B.9.2.1.1/1 [redacted] 2014a BAS 750 F Acute toxicity study in the rainbow trout (*Oncorhynchus mykiss*) 2014/1036951
Guidelines:	EPA 72-1, EPA 850.1075, OECD 203 (1992)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8% ± 1.0%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*) approximately 4 months old with a mean body length for control fish of 6.0cm (5.5 cm-6.6cm) and a mean wet weight for control fish of 1.68 g (1.17 g-2.43 g). The fish were supplied by Forellenzucht Trostadt GbR, Trostadt, Germany.

Test design: Flow through system (96 h) for 2 replicates per treatment with 10 fish per aquarium (loading 0.37 g fish/L/day) and 14 days acclimatisation. An assessment of mortality and sub-lethal effects occurred within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Due to low solubility of the test substance, test concentrations were dilutions of a saturated solution of BAS 750 F. Consequently it was not possible to set nominal concentrations, but instead represent the results as percentages of the saturated solution. The test solutions were a control (dilution water), 4.6, 10, 22, 46 and 100% corresponding to mean measured concentrations of <LOQ, 0.069, 0.142, 0.380, 0.826 and 1.55 mg a.s./L. All test treatments were visibly clear over the entire exposure period and no undissolved material was observed.

Preparation: Saturated stock solution was prepared using saturation columns in parallel to achieve and maintain saturated test concentrations.

Test conditions: 9L glass aquaria with a test volume of 9L, dilution water of charcoal-filtered drinking water mixed with deionised water, no aeration and no feeding.

Flow rate:	31.3 mL/min;
Hardness:	approximately 100 $CaCO_3$ mg/L;
Temperature:	13-14°C;
pH	7.8-8.1;
Oxygen content:	7.5 mg/L-8.9 mg/L;
Conductivity:	197µS/cm;
Photoperiod	16h light: 8h dark;
Light intensity:	60-585lux.

Analytics: Analytical verification of test substance concentrations was conducted using a HPLC-method with MS detection. Limit of quantification 0.001 mg/L.

Statistics: Probit analysis for calculation of LC_{50}. SAS version 8.2 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen (minimum 80%)
- Test substance maintained within ±20% of the initial concentration (since nominal values were not being used, minimum recovery 95%)

The analysed contents of BAS 750 F ranged from 95% to 101% of mean measured concentrations at test initiation, from 100% to 104% after 48h and from 95% to 103% of mean measured concentrations at test termination. Thus, the measured concentrations of the test substance in the test solutions during the exposure period were within ± 20% of the overall mean measured value. The following biological results are based on mean measured concentrations.

After 96 hours of exposure, no mortality was observed in the dilution water control and at test substance concentrations of up to and including 0.142 mg a.s./L, whereas 5% mortality was observed at 0.380 mg a.s./L. At the two highest tested concentrations, all fish were dead after 96 hours of exposure. Sub-lethal effects were observed at 0.380 mg a.s./L after 96 hours, and apathy was noted in the two highest concentrations before all the test organisms died. The results are summarised in Table B.9.2.1.1/1-1.

Table B.9.2.1.1/1-1: Acute toxicity (96 h) of BAS 750 F to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (mean measured)	**--**	**0.069**	**0.142**	**0.380**	**0.826**	**1.55**
Mortality [%] (96 h)	0	0	0	5	100	100
Symptoms (after 96 h) *	none	none	none	5D	n.d.	n.d.
Endpoints [mg BAS 750 F/L] (mean measured)						
LC_{50} (96 h)	0.532 (95% confidence limits: 0.47-0.61)					
NOEC (96 h)	0.142					

n.d.=not determined; all fish dead

* Symptoms after 96 h: D=swimming at the bottom.

III. CONCLUSION

In a flow-through acute toxicity study with rainbow trout the LC_{50} (96 h) of BAS 750 F was 0.532 mg a.s./L based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC_{50} of 0.532 mg a.s./L. The body length of the test subjects was 5.5-6.6cm, exceeding the recommended body length for this species (5.0±1.0cm). However as this was the only deviation, all the validity criteria were met and no adverse effects were observed, the study is still considered acceptable. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:

LC_{50} of 0.532 mg a.s./L (mm)

Report:	B.9.2.1.1/2 [redacted] 2015c BAS 750 F Acute toxicity study in the common carp (*Cyprinus carpio*) 2015/1249071
Guidelines:	EC 440/2008 C.1 Acute Toxicity for Fish, OECD 203, EPA 72-1, EPA 850.1075
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F (Reg. no.: 5834378), batch no. COD-001740, purity: 98.8% ± 1.0%.

B. STUDY DESIGN

Test species: Common carp (*Cyprinus carpio*) approximately 5 months old with a mean body length for control fish of 3.9cm (3.6 cm-4.0cm), a mean body weight for control fish of 0.81 g (0.56 g-1.11 g) and were supplied by Osage Catfisheries, Inc., Osage Beach, MO, USA.

Test design: Flow through system (96 h) with 5 test substance concentrations plus a dilution water control, 2 replicates per treatment and 10 fish per aquarium (loading 0.18 g fish/L/day). Assessment of mortality and sub-lethal effects within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure. Because the test substance is poorly water soluble a saturated stock solution was prepared by using saturation columns.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Due to low solubility of the test substance, test concentrations were dilutions of a saturated solution of BAS 750 F. Consequently it was not possible to set nominal concentrations, but instead represent the results as percentages of the saturated solution. The test solutions were a control (dilution water), 4.6, 10, 22, 46 and 100% of a saturated solution of BAS 750 F corresponding to mean measured concentrations of 0.082, 0.171, 0.414, 0.812 and 1.57 mg a.s./L. Test solutions were visibly homogenous.

Preparation: Saturated stock solutions prepared from two columns in parallel of 9.06135 and 9.07814g with approximately 200 ml of acetone each.

Test conditions: Stainless steel aquaria (29×21×22cm), test volume: 9L; test water: non-chlorinated, charcoal-filtered drinking water mixed with deionised water; flow rate: min. 1.88 L/h (min. 5 volume exchanges per day); hardness: 1.11mmol/L; temperature: 23°C; pH 8.0-8.1; oxygen content: 7.5 mg/L-8.0 mg/L; conductivity: 248µS/cm; photoperiod 16h light: 8h dark; light intensity: approximately 80-328lux; no aeration; no feeding.

Analytics: Analytical verification of test substance concentrations was conducted using a HPLC-method with MS detection. The limit of quantification was 0.001 mg/L.

Statistics: Probit analysis for calculation of LC_{50}. ToxRat Professional 2.10 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen (>75%)
- Test substance maintained within ±20% of the nominal values (minimum 89%)

Analytical verification of BAS 750 F concentrations was conducted in each test substance concentration at the beginning of the test, after 48h and at the end of the test. The mean measured concentrations in the test substance treatments were 0.082, 0.171, 0.414, 0.812 and 1.57 mg BAS 750 F/L. The analysed contents of BAS 750 F ranged from 107% to 116% of mean measured concentrations at test initiation, from 89% to 102% after 48h and from 91% to 103% of mean measured concentrations at test termination. Thus, the measured concentrations of the test substance in the test solutions during the exposure period were within ± 20% of the overall mean measured values. The following biological results are based on mean measured concentrations.

After 96 hours of exposure, no mortality was observed in the control and at test substance concentrations of up to and including 0.812 mg a.s./L, whereas 100% mortality was observed at 1.57 mg a.s./L. No sub-lethal effects were found in the control and at test substance concentrations of up to and including 0.812 mg a.s./L. The results are summarised in Table B.9.2.1.1/2-1.

Table B.9.2.1.1/2-1: Acute toxicity (96 h) of BAS 750 F to common carp (*Cyprinus carpio*)

Concentration [mg a.s./L] (mean measured)	**--**	**0.082**	**0.171**	**0.414**	**0.812**	**1.57**
Mortality [%] (96 h)	0	0	0	0	0	100
Symptoms (after 96 h)	none	none	none	none	none	n.d.
Endpoints [mg BAS 750 F/L] (mean measured)						
LC_{50} (96 h)	1.126 (95% confidence limits: n.c.)					
NOEC (96 h)	0.812					

n.d.=not determined; all fish dead; n.c.=not calculated due to mathematical reasons/inappropriate data

III. CONCLUSION

In a flow-through acute toxicity study with common carp the LC_{50} (96 h) of BAS 750 F was 1.126 mg a.s./L based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC_{50} of 1.126 mg a.s./L. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
LC_{50} of 1.126 mg a.s./L (mm)

Report:	B.9.2.1.1/3 [redacted] 2015a BAS 750 F (Reg.No. 5834378) Zebra fish acute toxicity test 2015/1001581
Guidelines:	OECD 203 (1992), EPA 850.1075
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F (Reg. no.: 5834378), batch no. COD-001740, purity: 98.8% ± 1.0%.

B. STUDY DESIGN

Test species: Zebrafish (*Brachydanio rerio*, syn. *Danio rerio*) approximately 6 months old with; a mean body length of 2.77cm ± 0.14 and a mean wet weight of 0.29 g ± 0.04. The fish are an in house culture, originally obtained from "The culture of fish in GoczalK_{OW}ice", Poland.

Test design: Static system (96 h) for 5 test substance concentrations plus a reconstituted water control with 2 replicates per treatment and 10 fish per aquarium (loading 0.29 g fish/L). Assessment of mortality and sub-lethal effects occurred at 3, 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (reconstituted water), the filtrate of a loading of 10 mg BAS 750 F /L, its 1.5, 2.25, 3.38, 5.06-fold dilution corresponding to geometric mean concentrations of 1.110, 0.913, 0.735, 0.593 and 0.475 mg a.s./L, respectively.

Preparations: The correct quantity of the test substance was added to each flask with test medium to 4.5L, and then sonicated for up to 15 minutes and mechanically shaken for 24h.

Test conditions: 10L glass aquaria; dilution water: reconstituted water; no aeration; no feeding.

Hardness:	242.04-264.44 mg $CaCO_3$/L;
Temperature:	24.0-24.4°C;
pH	7.60-7.76;
Oxygen content:	80.7-96.2%;
Conductivity:	630-648µS/cm;
Photoperiod:	16h light: 8h dark with 30 min transition;

Analytics: Analytical verification of test substance concentrations was conducted using a LC-method with DAD detection. The limit of quantification was 0.01 mg/L and the limit of detection was 0.005 mg/L.

Statistics: Probit analysis for calculation of LC_{50}. ToxRat Professional 2.10 was used to perform the statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen (minimum 80.7%)
- Test substance maintained within ±20% of the initial concentration (minimum recovery 51.25%)

Analytical verification of BAS 750 F concentrations was conducted in each test substance concentration at the beginning of the test, after 48h and at the end of the test. The concentrations determined in samples collected at exposure initiation were 1.566 and 1.511 mg/L in the filtrate of a loading of 10 mg/L, 1.094 and 1.097 mg/L in its 1.5-fold dilution, 0.713 and 0.676 mg/L in its 2.25-fold dilution, 0.492 and 0.461 mg/L in its 3.38-fold dilution, 0.331 and 0.320 mg/L in its 5.06-fold dilution. The concentrations of test substance determined in samples collected after 48h of exposure were in the range of 60.73-79.59% of initial concentrations. The concentrations of test substance determined in samples collected at exposure termination were in the range of 51.25-69.16% of initial concentrations. Therefore the following biological results are based on geometric mean concentrations.

After 96 hours of exposure, no mortality was observed in the control and at test substance concentrations of up to and including 0.593 mg a.s./L, whereas 5% and 45% mortality was observed at 0.735 and 0.913 mg a.s./L, respectively. At the highest tested concentration, all fish were dead after 96 hours of exposure. Sub-lethal effects were found at 0.0.735 and 0.913 mg a.s./L after 96 hours. The results are summarised in Table B.9.2.1.1/3-1.

Table B.9.2.1.1/3-1: Acute toxicity (96 h) of BAS 750 F to zebrafish (*Brachydanio rerio*)

Concentration [mg a.s./L] (geometric mean)	**Control**	**0.475**	**0.593**	**0.735**	**0.913**	**1.110**
Mortality [%] (96 h)	0	0	0	5	45	100
Symptoms (after 96 h) *	none	none	none	1U	11U, 11F	n.d.
Endpoints [mg BAS 750 F/L] (geometric mean measured)						
LC_{50} (96 h)	0.906 (95% confidence limits: 0.857-0.954)					
NOEC (96 h)	0.593					

n.d.=not determined; all fish dead

* Symptoms after 96 h total over two replicates of 10 fish: U=unbalanced swimming behavior; F=faulty respiratory function,h=haemorrhaging, D=discolouration

III. CONCLUSION

In a static acute toxicity study with zebrafish the LC_{50} (96 h) of BAS 750 F was 0.906 mg a.s./L based on geometric mean concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC_{50} of 0.906 mg/L. The RMS notes that the hardness and the conductivity exceeded the recommended limit of 250 mg $CaCO_3$/L and 10 μScm^{-1} respectively, although as all the validity criteria were met and no negative effects were observed in the controls, this deviation is not expected to have adversely affected the experiment. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
LC_{50} of 0.906 mg/L (mm)

Report:	B.9.2.1.1/4
	[redacted] 2014a
	BAS 750 F: Acute toxicity to the sheepshead minnow, *Cyprinodon variegatus*, determined under static-renewal test conditions
	2014/7002810
Guidelines:	EPA 850.1075
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F (Reg. no.: 5834378), batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Juvenile sheepshead minnow (*Cyprinodon variegatus*) with mean body length for control fish of 17 ± 2.4mm, mean wet weight for control fish of 0.1388 ± 0.0579 g and supplied by "Aquatic BioSystems", Fort Collins, Colorado, USA.

Test design: Semi-static system (96 h) with renewal of test solutions 48 hours after test initiation, 5 test substance concentrations plus a dilution water control and a solvent control, 2 replicates per treatment and 10 fish per aquarium (loading 0.1735 g fish/L). Assessment of mortality and sublethal effects occurred at 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.18, 0.35, 0.70, 1.4 and 2.7 mg BAS 750 F/L (nominal), corresponding to mean measured concentrations of 0.131, 0.258, 0.517, 1.12 and 2.05 mg a.s./L. All test solutions were clear and colourless with no visible precipitate, film or undissolved test substance.

Preparation: 0.27g of BAS 750 F was added to 10 ml of DMF and then serially diluted

Test conditions: 10L glass aquaria, test volume: 8L; dilution water: laboratory saltwater (prepared by mixing a commercial sea salt mix to laboratory freshwater), no aeration; no feeding.

Salinity:	19.7-19.9 ‰;
Temperature:	21.8-22.6°C;
pH:	8.0-8.1;
Oxygen content:	7.4 mg/L -7.6 mg/L (100-103% fresh solutions), 6.0 mg/L-7.2 mg/L (79-95% spent solutions);
Photoperiod:	16h light: 8h dark;
Light intensity:	956lux;

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with MS/MS-detection. The minimum quantification limit was 0.00250 mg/L.

Statistics: Probit analysis for determination of the LC_{50} value, Dunnett's one-tailed t-test and one-tailed Fisher`s t-test with Hochberg's family-wise correction for determination of the NOEC. SAS version 9.3 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen (minimum 91%)
- Test substance maintained within ±20% of the nominal values (minimum 69%)

Analytical verification of BAS 750 F concentrations in fresh and old solutions was conducted in all concentrations at test initiation, after 48 hours and at test termination. Samples of the highest test substance concentration were not analysed beyond the samples of the old solution after 48 hours due to 100% mortality. The analysed contents of BAS 750 F in new solutions ranged from 76 to 79% of nominal and the measured concentrations in old solution were between 69% and 81% of nominal. The following biological results are based on mean measured concentrations.

After 96 hours of exposure, no mortality was observed in the water control and the solvent control, whereas 5% mortality occurred at 0.131 mg a.s./L, however, this mortality was not considered biologically significant, as no mortality was observed in the two higher test substance concentrations of 0.258 and 0.517 mg a.s./L. All fish were dead at the two highest test substance concentrations after 96 hours of exposure. Sublethal effects were observed at 0.258 and 0.517 mg a.s./L after 96 hours. The results are summarised in Table B.9.2.1.1/4-1.

Table B.9.2.1.1/4-1: Acute toxicity (96 h) of BAS 750 F to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.18**	**0.35**	**0.70**	**1.4**	**2.7**
Concentration [mg a.s./L] (mean measured)	**Control**	**Solvent control**	**0.131**	**0.258**	**0.517**	**1.12**	**2.05**
Mortality [%] (96 h)	0	0	5 [a)]	0	0	100	100
Symptoms (after 96 h) [#]	none	none	none	1S, 1LE, 1DC	20DC, 2B, 1LE,	n.d.	n.d.
Endpoints [mg BAS 750 F/L] (mean measured)							
LC_{50} (96 h)	0.761 (95% confidence limits: n.c.)						
NOEC (96 h)	0.131						

[a)] This mortality was not considered biologically significant as compared to the control.
[#] Symptoms after 96 h: B=on bottom, DC=discoloration, LE=loss of equilibrium, S=surfacing.
n.d.=not determined; all animals dead
n.c.=not calculated

III. CONCLUSION

In a semi-static acute toxicity study with sheepshead minnow the LC_{50} (96 h) of BAS 750 F was 0.761 mg a.s./L based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC_{50} of 0.761 mg a.s./L. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
LC_{50} of 0.761 mg a.s./L (mm)

B.9.2.1.2 Acute toxicity to fish from metabolites

Report:	B.9.2.1.2/1 [redacted] 2015b Reg.No. 6003432 (metabolite of BAS 750 F, M750F007)-Rainbow trout, acute toxicity test 2015/1001489
Guidelines:	OECD 203 (1992), EPA 850.1075
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F007 (metabolite of BAS 750 F), batch no. L87-32-1, purity: 97.0%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* Walbaum) age approximately 7 months old, body length of 5.7 ± 0.3 cm, body weight of 2.25 g ± 0.32 and supplied by 'The culture of salmonidae Fish in Zawoja', Poland.

Test design: Static system (96 hours) limit test with two replicates with 10 fish per aquarium (loading 0.75 g fish/L). Assessment of mortality and symptoms of toxicity occurred within 3 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, solvent control (DMF), filtrate of a loading of 10 mg M750F007/L, corresponding to a geometric mean measured concentration of 7.2 mg M750F007/L. All test solutions were visually homogenous, colourless and transparent without any undissolved particles.

Test substance preparation: The stock test solution was created with 15 minutes of sonication and filtration through a 0.45µ nitrocellulose membrane.

Test conditions: Glass aquaria, test volume 30L of reconstituted water and no feeding;

Temperature:	13.4°C -14.3°C;
pH	7.18-7.80;
Oxygen saturation:	91%-99%;

Total hardness:	246.71-247.37 mg $CaCO_3$/L;
Conductivity:	635-659µS/cm;
Photoperiod:	16h light: 8h dark with 30 min transition

Analytics: Analytical verification of test substance concentrations was conducted using a LC-method with DAD detection. The limit of quantification was 0.002 mg/L and the limit of detection was 0.0005 mg/L.

Statistics: No analysis necessary.

II. RESULTS AND DISCUSSION

All the validity criteria were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen (minimum 91%)
- Test substance maintained within ±20% of the initial concentration (since nominal values were not being used, minimum recovery 92.33%)

Analytical verification of test substance concentrations was conducted in each replicate at test initiation, after 48h of exposure and at test termination. At exposure initiation the determined concentrations of test substance in the filtrate of a loading of 10 mg/L were 7.38 mg/L and 7.43 mg/L in replicates A and B, respectively. After 48h of exposure the determined concentrations of test substance were 7.37 mg/L and 7.19 mg/L, respectively. At exposure termination determined concentrations of test substance were 7.24 mg/L (98.10% of initial concentration) and 6.86 mg/L (92.33% of initial concentration), respectively. The geometric mean concentration of the test substance in the filtrate of a loading of 10 mg/L was 7.20 mg/L. The following biological results are based on geometric mean measured concentrations.

No mortality or other symptoms of toxicity were observed after 96 hours of exposure in the control groups and at 7.2 mg M750F007/L. The results are summarised in Table B.9.2.1.2/1-1.

Table B.9.2.1.2/1-1: Acute toxicity (96 h) of M750F007 (metabolite of BAS 750 F) on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (geometric mean measured)	**Control**	**Solvent control**	**7.2**
Mortality [%]	0	0	0
Symptoms	none	none	none
Endpoints [mg M750F007/L] (geometric mean measured)			
LC_{50} (96 h)	> 7.2		
NOEC (96 h)	≥ 7.2		

III. CONCLUSION

In a static acute toxicity study (limit test) with rainbow trout, the LC_{50} (96 h) of M750F007 (metabolite of BAS 750 F) was determined to be >7.2 mg/L based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC_{50} of >7.2 mg/L. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
LC_{50} of >7.2 mg/L (mm)

Report:	B.9.2.1.2/2 [redacted] 2016 Reg. No. 5863469 (Metabolite of BAS 750 F, M750F006) Rainbow trout, Acute toxicity test 2016/1128152
Guidelines:	OECD 203 (1992), EPA 850.1075
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F006 (metabolite of BAS 750 F), batch no. L85-170, purity: 95.6%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* Walbaum), aged approximately 3 months old with a body length of 4.44±0.14 cm and a body weight of 0.94 ± 0.11g, supplied by 'The culture of salmonidae Fish in Zawoja', Poland.

Test design: Static system (96 hours) with two replicates with 10 fish per aquarium (loading 0.94 g fish/L). Assessment of mortality and symptoms of toxicity were performed at 3, 6, 24, 48, 72 and 96h.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, solvent control (DMF), loadings with a nominal concentration of 12, 6, 3, 1.5 and 0.75 mg/L. All test solutions were visually homogenous, colourless and transparent without any undissolved particles.

400.4 mg of the test substance were weighed into a glass vial and 4000 µL of DMF (N,N-dimethyl formamide, an organic solvent) were added and mixed by inversion to form a stock solution. Test solutions were prepared by dilution from the stock solution, sonicated for 15 minutes then passed through a 0.45µm nitrocellulose membrane filter.

Test conditions: Ten litre glass aquaria with reconstituted water and no feeding;

Temperature:	14.1-15.1°C;
pH	6.81-7.05;
Oxygen saturation:	94-100%;
Total hardness:	231.04-246.64 mg $CaCO_3$/L;
Conductivity:	580-590µS/cm;
Photoperiod:	16h light: 8h dark with 30 min transition

Analytics: The concentrations of the test substance were chemically determined using a validated method of high performance liquid chromatography with DAD detection. The limit of quantification was 0.005 mg/L and the limit of detection was 0.001 mg/L.

Statistics: Spearman-Kärber method for determination of LC_x value, Step-down Cochran-Armitage Test for LOEC and NOEC determination. Fisher's Exact

Binomial Test was used for comparison of the solvent control with the control. ToxRat Professional was the software used to perform statistical calculations.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 203 (1992)):

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen (94-101%)

The concentrations of the test substance were determined using a validated method in samples of every treatment from each aquarium at exposure initiation, after 48 h of exposure and at exposure termination. Recoveries of the test substance were 77.3-97.9% at test termination compared to the nominal concentrations of the filtrates, and therefore pass out of ±20% from the nominal concentrations. Therefore, the following biological results are based on geometric mean measured concentrations. The geometric mean concentrations of the test substance were 9.78, 5.09, 2.70, 1.48 and 0.78 mg/L, respectively.

Mortality is presented in Table B.9.2.1.2/2-1 and symptoms of intoxication in Table B.9.2.1.2/2-2. No significant difference between the solvent control and the control was detected (Fisher's Exact Binomial Test, p=0.05); therefore, the solvent control was used for further statistical analyses. The median concentration causing 50% mortality of carp after 96 hours of exposure LC_{50} is 6.20 mg/L (95% confidence interval: 5.27 – 7.30 mg/L).

At exposure termination there was a significant difference between the geometric mean test substance concentrations 5.09 mg/L, 9.78 mg/L and the solvent control for mortality. Therefore, the lowest geometric mean test substance concentration causing mortality, i.e. the LOEC, is 5.086 mg/L and the geometric mean test substance concentration not causing effect on fish survival the NOEC value is 2.696 mg/L.

Table B.9.2.1.2/2-1: Acute toxicity of M750F006 (metabolite of BAS 750 F) on rainbow trout (*Oncorhynchus mykiss*)

Geometric mean measured concentration (mg/L)	Number of fish tested	Number of mortalities						Total dead at exposure termination (%)
		3h	6h	24h	48h	72h	96h	
Control	10	0	0	0	0	0	0	0
Solvent control	10	0	0	0	0	0	0	0
0.775	10	0	0	0	0	0	0	0
1.478	10	0	0	0	0	0	0	0
2.696	10	0	0	0	0	0	0	0
5.086	10	0	0	0	0	0	2	20
9.777	10	0	0	10	10	10	10	100

Table B.9.2.1.2/2-2: Symptoms of intoxication of M750F006 (metabolite of BAS 750 F) on rainbow trout (*Oncorhynchus mykiss*)

Geometric mean measured concentration (mg/L)	Symptoms observed					
	3h	6h	24h	48h	72h	96h
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
0.775	0	0	0	0	0	0
1.478	0	0	0	0	0	0
2.696	2 ES	2 ES	2 ES	2 ES	2 ES	2 ES
5.086	3 ES, 3 H	3 ES, 3 D, 3 H, 3 OE	5 ES, 5 D, 5 H, 3 OE	10 ES, 1 LR, 1 L, 8 D, 8 H, 8 OE	10 ES, 2 LR, 2 L, 8 D, 8 H, 8 OE	8 ES, 6 D, 6 H, 6 OE[1]
9.777	7 ES, 7 LR, 7 D, 7 H	10 ES, 10 LR, 4 L, 10 D, 10 H, 4 OE	-	-	-	-

[1] Two mortalities had occurred at this concentration and time

Symptoms: ES – Erratic swimming LR – Loss of reflex L – Lethargy
D – Discolouration H – Hyperventilation OE – Opaque eyes

III. CONCLUSION

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of M750F006 (metabolite of BAS 750 F) was determined to be 6.20 mg/L based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an LC_{50} of 6.20 mg/L. Given that signs of treatment related intoxication were observed at 2.696 mg/L (Table B.9.2.1.2/2-2) the LOEC and NOEC should be 2.696 and 1.478 mg/L respectively. The RMS notes that the conductivity of 580-590µS/cm exceeds the recommended conductivity of ≤10µS/cm in OECD 203 (1992). Given all the validity criteria were met and no adverse effects were observed in the controls, this deviation is not expected to have negatively affected the experiment. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
LC_{50} of 6.20 mg/L (mm)

B.9.2.2. Long-term and chronic toxicity to fish

Report: B.9.2.2/1
[redacted] 2015 a
BAS 750 F: Early life-stage toxicity test with the sheepshead minnow, *Cyprinodon variegatus*, under flow-through conditions
2015/7000619
Guidelines: EPA 850.1400
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F; batch no. COD-001740; purity: 98.8%.

B. STUDY DESIGN

Test species: Sheepshead Minnow (*Cyprinodon variegatus*); newly fertilised eggs (< 24 hours post-fertilization) obtained from in-house culture.

Test design: Flow-through system (35 d) for 5 test substance concentrations plus a dilution water control and a vehicle control with 4 replicate test chambers per treatment with 20 fertilised eggs in each. A proportional diluter system was used for the preparation of test solutions and intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. On a daily basis during incubation, the embryos were counted and dead embryos were removed and discarded. On study day 11 (day 4 post-hatch), all live fry were counted and released into their respective replicate growth chamber. Survival of the fry was monitored daily, and any behavioural or physical changes were recorded, including abnormalities. On day 35 (28 days post hatch) surviving animals were sacrificed and measured for length and weight.

Endpoints: NOEC values based on hatchability, survival, toxic signs and growth.

Test concentrations: Control (dilution water), vehicle control (0.05 mL DMF/L), 0.010, 0.020, 0.040, 0.080, and 0.16 mg a.s./L (nominal). All test solutions were clear and colourless with no visible particulates, films or undissolved test substance.

Preparation: Test solutions were prepared by serial dilution a stock solution periodically prepared by diluting 0.3239g (0.32g corrected for purity) of a.s. to a volume of 0.1L with DMF.

Test conditions: Test vessels were glass aquaria (15×21.5×24cm) with a test volume of approximately 5.0L and one glass incubation cup per test vessel (used during embryo stage) with 9cm diameter and Nitex® screen replacing the bottom. The test medium was commercial sea salt mix (Crystal Sea Marine Mix, Marine Enterprise International, Inc. Baltimore, Maryland) added to demineralized, filtered and sterilized laboratory freshwater. Fish larvae were initially fed three times daily *ad libitum* with brine shrimp nauplii (*Artemia*), and standard commercial fish food was added to the daily food beginning on day 21. No aeration was performed during the test.

Temperature:	24.6-25.9°C;
pH:	7.9-8.2;
Dissolved oxygen:	5.6 mg/L-7.7 mg/L;
Salinity:	19.6-20.1‰;
Light intensity:	483-602lux;
Photoperiod:	16h light: 8h dark (two 30-minute transitions);
Flow rate:	7.2 volume additions per test chamber per 24h;

Analytics:	Analytical verification of BAS 750 F concentrations was conducted using an HPLC-method with MS/MS detection. The minimum quantifiable limit was 0.00250 mg/L.
Statistics:	Fisher's exact test and t-test for comparison of controls; Fisher's exact test and/or ANOVA followed by one-tailed Dunnett's test (α=0.05) for determination of NOEC. SAS version 9.3 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 210 (2012)):

- Water temperature should not differ by more than ±1.5°C (24.6-25.9°C)
- ≥60% dissolved oxygen (>75%)
- ≥70% control hatching success (91%)
- ≥75% control post-hatch success (97%)

Analytical measurements of BAS 750 F concentrations were conducted in samples collected three days prior to initiation and in the control and in all test substance concentrations at test initiation, on study days 0, 7, 11, 14, 21, 28 and at test termination after 35d of exposure. The mean measured concentrations of BAS 750 F in the test-substance treatments for the 35-day exposure ranged from 86% to 92% of the nominal concentrations. Measured concentrations of BAS 750 F in the test substance treatments prior to initiation of the definitive test ranged from 81% to 84% of the nominal concentrations. Measured concentrations of BAS 750 F in the test substance treatments on days 0 through 35 were between 82% and 96% of nominal and therefore for all test substance treatments, exposures were maintained within 20% of the nominal. The following biological results are based on mean measured concentration.

Egg hatch began on day 5 or 6 and ended between study days 6 and 11 in the control and all test substance treatments. 95% hatch was reached in all treatments by study day 9 (although 95% hatch was on day 7 for the control, then day 0 post-hatch) and the overall hatching success in the control and vehicle control was 91 and 93%, respectively. Post-hatch survival was 97 and 93% in the control and vehicle control, respectively and between 94 and 97% in all test substance treatments. No statistically significant effects on the hatching success, post-hatch survival, time to start of hatch and time to end of hatch was observed for any of the test substance treatments as compared to the control.

The mean standard fish lengths in the test substance treatments ranged from 14.0mm to 14.7mm compared to 14.1mm in the control and 14.2mm in the vehicle control treatments. Mean blotted wet weight in the control and vehicle control was 0.0836 g and 0.0887 g, respectively. Mean blotted wet weight in the test substance treatments ranged from 0.0826 g to 0.0969 g. There was no statistically significant reduction in length and blotted wet weight in any of the test substance treatments as compared to the control.

The only morphological or behavioural abnormalities observed during the exposure were fish on the bottom of the test chamber and spinal curvature. Fish on the bottom of the test chamber was observed for one fish in the 0.147 mg a.s./L test treatment on study days 12 and 13. Spinal curvature was observed for one fish in the vehicle control on study days 18 and 19. These abnormalities were judged not to be test substance related because no concentration-response relationship was evident. No other morphological or behavioural abnormalities were noted. The results are summarised in Table B.9.2.2/1-1.

Table B.9.2.2/1-1: Chronic toxicity of BAS 750 F to sheepshead minnow (*Cyprinodon variegatus*) in a fish early life-stage test (35 d)

Concentration [mg a.s./L] (nominal)	**Control**	**Vehicle control**	**0.010**	**0.020**	**0.040**	**0.080**	**0.16**
Concentration [mg a.s./L] (mean measured)	**<LOQ**	**<LOQ**	**0.00861**	**0.0172**	**0.0356**	**0.0725**	**0.147**
Hatching success [%]	91	93	96	84	88	90	88
Start of hatch [d]	6	6	6	6	6	6	6
Time to 95% hatch [d]	7	8	7	8	7	7	7
End of hatch [d]	7	9	7	8	7	7	7
Post-hatch survival [%] on day 35	97	93	96	97	94	97	97
Mean standard length on day 35 [mm] (standard deviation)	14.1 (1.2)	14.2 (1.4)	14.2 (0.72)	14.4 (1.3)	14.7 (0.85)	14.1 (1.3)	14.0 (1.3)
Mean blotted wet weight on day 35 [g] (standard deviation)	0.0836 (0.0193)	0.0887 (0.0198)	0.0969 (0.0818)	0.0859 (0.0293)	0.0911 (0.0158)	0.0826 (0.0202)	0.0882 (0.0215)
Endpoints [mg BAS 750 F/L] (mean measured concentration)							
$NOEC_{overall}$ (35 d)	**0.147**						

The NOEC is given as primary endpoint, since no dose-response relationship was derived from the study which could be used for EC_x calculations.

III. CONCLUSION

In an early life stage study with sheepshead minnow (*Cyprinodon variegatus*), the overall NOEC (35 d) for BAS 750 F was determined to be 0.147 mg a.s./L, based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is a NOEC (35 d) of ≥0.147 mg a.s./L. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
NOEC (35 d) of 0.147 mg a.s./L (mm)

Report: B.9.2.2/2
[redacted] 2015a
BAS 750 F- Early life-stage toxicity test on the zebrafish (*Danio rerio*) in a flow through system
2014/1262160
Guidelines: OECD 210, EPA 72-4 (a), EPA 850.1400, EPA 540/9-86-138
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD 001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Zebrafish (*Danio rerio*) eggs fertilised less than 6 hours to test initiation, sourced from in-house rearing.

Test design: Flow through system with 5 test substance concentrations plus a dilution water control, 4 replicates per treatment with 25 fertilised eggs in each. Eggs and larvae were exposed in cylindrical glass vessels (egg cups); surviving larvae were released to their respective test vessels on day 20. The test solution flowed continuously from the mixing tank into an "udder" which split the test water into 4 equal parts for the 4 replicate test aquaria. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behaviour. On day 36, fish were sacrificed and the body length and weight of surviving individuals were determined.

Endpoints: NOEC values based on hatch rate, post-hatch survival, toxic signs, growth and time spans to hatch and swim-up.

Test concentrations: Due to low solubility of the test substance, test concentrations were dilutions of a saturated solution of BAS 750 F. Consequently it was not possible to set nominal concentrations, but instead represent the results as percentages of the saturated solution. The test solutions were a control (dilution water), 0.010, 0.024, 0.060, 0.150 and 0.375 mg BAS 750 F/L (nominal), corresponding to mean measured concentrations of <LOQ, 0.011, 0.027, 0.063, 0.172 and 0.444 mg a.s./L. The stock solution was visible clear and colourless and the homogeneity of the test solutions was demonstrated by filtration and comparison to un-filtered stock being <20%.

Preparation: Saturated stock solution using saturation columns of 11g of a.s. dissolved within 200 ml acetone, each for up to 14 days (generally two in parallel with a new column prepared once per week). Throughout the exposure period the stock solution was continuously diluted with aerated dilution water at a constant rate, and the mixture flowed into four test vessels via a flow splitter.

Test conditions: The test vessels were stainless steel aquaria (29×21×22cm), volume 9L with egg cups (cylindrical glass vessels, diameter 19cm and volume 1.7 L). The dilution water was non-chlorinated, filtered tap water (diluted with deionized water); slight aeration from day 24 on

Temperature:	23.8-25.5°C;
pH:	7.8-8.3;
Oxygen content:	6.1 mg/L-8.3 mg/L;
Hardness:	1.11-1.23mmol/L;
Conductivity:	277-282µS/cm;
Acid capacity:	2.59mmol/L.
Light intensity:	73-148lux;
Photoperiod:	16 hours light: 8 hours dark;

	Flow rates: 2.25 L/hour/test vessel equal to an approximately 6-fold exchange over 24h.
	Feeding: freshly hatched *Artemia nauplii* and commercial fish diet (Tetramin, supplied by Tetra-Werke; Seramicron, supplied by Sera) from day 7 on.
Analytics:	Analytical verification of test substance concentrations was conducted using an HPLC-method with MS detection with test solutions from alternative replicates (except on day 0 where samples were taken from all replicates). Additional samples were taken where any deviation was >20%. Samples were taken from the middle of the vessel. The limit of quantification was 0.001 mg/L.
Statistics:	One-sided Jonkheere-Terpstra for embryo, larvae and juvenile fish survival, one-sided William´s test for weight and length data and one-sided Wilcoxon-test for variability between replicates. SAS version 9.3 was used.

II. RESULTS AND DISCUSSION

The following are the validity criteria for the study (OECD 210 (2013)):

- >60% dissolved oxygen concentration (minimum 73%)
- No more than ±1.5°C difference in water temperature (not met, 23.8-25.5°C)
- Analytical measurement of test concentrations (95.4-126.1% of nominal)
- ≥70% hatching success (98%)
- ≥75% post-hatch success (100%)

Analytical verification of test substance concentration was conducted in all test substance concentrations at weekly intervals until day 36. Mean measured concentrations of BAS 750 F ranged from 95.4% to 126.1% of nominal over the exposure period. The following biological results are based on mean measured concentrations.

Hatching started simultaneously in all test groups on day 3 and was complete by day 6. No test substance-related effect was observed on the time to start or end of hatching. Hatching success ranged from 96-100% for the replicates of the control group and there was no statistically significant decrease in hatching success in any of the treatment groups in comparison to the control group. Survival from hatch to the end of swim-up (day 7) was 100% for all replicates of the control group and there was no statistically significant decrease in survival in any of the treatment groups in comparison to the control group.

In all test groups, larval swim-up started on day 5 of exposure and was complete simultaneously with the control on day 7. No test substance-related effect was observed on the time to swim-up. From the end of swim-up to the end of exposure (day 7–36) survival was 96-100% for the replicates of the control group. The survival from the end of swim-up to the end of exposure (day 7–36) as well as the overall survival (day 0–36) was statistically significantly decreased in the treatment groups of 0.172 and 0.444 mg a.s./L in comparison to the control group.

There were no signs of toxicity or abnormalities observed among the replicates of the control group and the treatment groups. In comparison to the control group the total body lengths of the surviving fish at the end of the exposure period were statistically significantly decreased in the test groups of 0.063, 0.172 and 0.444 mg a.s./L (one-sided William´s test, $p \leq 0.05$ and $p \leq 0.01$). In comparison to the control group, the mean wet weights of the surviving fish at the end of the exposure period were statistically significantly decreased in the test groups of 0.172 and 0.444 mg a.s./L (one-sided William´s test, $p \leq 0.05$ and $p \leq 0.01$). The results are summarised in Table B.9.2.2.1-2.

Table B.9.2.2/2-1: Chronic toxicity of BAS 750 F to zebrafish (*Danio rerio*) in a fish early life stage test (36 d)

Concentration (nominal) [mg a.s./L]	**Control**	**0.010**	**0.024**	**0.060**	**0.150**	**0.375**
Concentration (mean measured) [mg a.s./L]	**<LoQ**	**0.011**	**0.027**	**0.063**	**0.172**	**0.444**
Embryo survival until hatch [%]	98	100	99	100	99	98
Survival of larvae from hatch until end of swim-up (day 7) [%]	100	100	100	99	100	100
Survival of young fish (day 7-36) [%]	99	96	98	96	**94** *	**95** *
Survival from day 0 to test termination (36 d) [%]	97	96	97	95	**93** *	**93** *
Start of hatch [day] End of hatch [day]	3 6	3 6	3 6	3 6	3 6	3 6
Start of swim-up End of swim-up	5 7	5 7	5 7	5 7	5 7	5 7
Symptoms	none	none	none	none	none	none
Mean weight (36 d) [mg]	48.1	51.4	47.3	44.2	**42.9 ****	**32.9 *****
% of control	--	106.7	98.3	91.9	89.1	68.4
Mean length (36 d) [cm]	1.76	1.74	1.71	**1.68 ****	**1.66 *****	**1.50 *****
% of control	--	99.2	97.6	95.9	94.3	85.6
	Endpoints [mg BAS 750 F/L] (mean measured)					
$NOEC_{overall}$ (36 d)	0.027					

Values printed in **bold** show statistically significant differences compared to the control.
* Statistically significant differences compared to the control (one-sided Jonkheere-Terpstra test, $p \leq 0.05$).
****** Statistically significant differences compared to the control (one-sided William´s test, $p \leq 0.05$).
******* Statistically significant differences compared to the control (one-sided William´s test, $p \leq 0.01$).

The NOEC is given as primary endpoint, since from the dose-response it is obvious that effects on survival or growth with ≥ 10% (EC_{10}, EC_{20}) occur only at higher test concentrations than the NOEC. The effect level of the most sensitive parameter (length) is less than 5% at the NOEC. Hence, the NOEC is protective and used as the most reliable endpoint for the risk assessment. EC_x were not calculated.

III. CONCLUSION

In an early life stage study with zebrafish (*Danio rerio*) the overall NOEC (36 d mean length) for BAS 750 F was determined to be 0.027 mg a.s./L based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is a NOEC (36d) of 0.027 mg a.s./L based on effects of mean fish length. The RMS notes that the validity criterion of temperature difference remaining within ±1.5°C was not met. However, the temperature minimum was according to a continuous temperature measurement rather than instantaneous which did not record any deviation. This would mean that the marginal passing was

temporary (45h) and may not have affected the test. This is supported by no negative effects being observed in the control organisms. Additionally the analytical results pass outside ±20% of the nominal, but the results were >100% of the nominal and the endpoint is based on mean measured concentration. Consequently, this failing of the validity criteria is not expected to have adversely affected the test and is therefore acceptable. The recommended duration of the test is 30 days post-hatch, although the study duration of 32 days post-hatch is not expected to have adversely affected the test. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
NOEC (36d) of 0.027 mg a.s./L (mm)

Report:	B.9.2.2/3 [redacted] 2015c ^{14}C-BAS 750 F (label: triazole-3(5)-C14)-Bioconcentration study in the rainbow trout (*Oncorhynchus mykiss*) 2015/1122811
Guidelines:	EPA 850.1730, OECD 305 (October 2012)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: Ratio of 2:1 radiolabelled and unlabelled test substance
Non-radiolabelled test substance: BAS 750 F, batch no. COD-001740, purity 98.8%.
Radiolabelled test substance: ^{14}C-BAS 750 F (label: triazole-3(5)-C14); batch no. 1062-2001; specific activity: 5.46 MBq/mg; chemical purity: 98.9%, radiochemical purity: 98.8%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*) body weight mean of 2.00 ±0.23 g and range 1.61-2.40g/fish and mean body length: 6.1 ± 0.2 cm. Fish were aged approximately 4 months and sourced from "Forellenzucht Trostadt GbR", Trostadt, Germany. Fish were acclimatised for 14 days prior to test initiation.

Test design: Flow-through system (14 days uptake, 7 days depuration) of one test vessel with 80 fish each (loading 0.32 g fish/L at initiation). Test substance concentrations in water and fish were determined 13 times over the study with radioactive residues measured separately in edible and nonedible portions, while the whole fish value was calculated from the weight normalized sum of the individually measured portions. Lipid content was determined gravimetrically after organic exctraction using a procedure based on the Smedes-method as recommended in OECD 305 (2012). Fish from control group were tested for lipid content at the start and end of uptake and at the end of depuration while mortality and signs of toxicity were assessed daily.

Endpoints: Bioconcentration potential (bioconcentration factors BCFss (mean value of $C_F(t)/C_W$), $BCF_{SSL,}$ BCF_K, $BCF_{Kg,}$ BCF_{KLg}); uptake rate; depuration rate; depuration half-life; time to 95% steady state.

Test concentrations: Control, 0.010 mg BAS 750 F/L (nominal). Homogeneity of the test solutions was demonstrated by filtration (92-96% of nominal) and comparison to unfiltered stock (95-97% of nominal). Radioactivity was tested in filtered and unfiltered samples, with the outcome that only unfiltered samples were used for subsequent analysis.

Preparation: The radiolabelled test substance was mixed with unlabelled substance in a ratio of 2:1 labelled: unlabelled. The stock solution was prepared from 400 µg/L [^{14}C] test substance and half the amount of unlabelled test substance. The weighing boat was rinsed with 20 ml of acetone into the vial of weighed test substance, then added to the empty stock solution tank with additional acetone rinses. The acetone was then left to evaporate, after which dilution water was added and the solution stirred for one day. Metering pumps delivered the stock solution into a mixing vessel with tempered dilution water, which is then delivered into the test vessel.

Test conditions: Silicon-sealed glass aquaria (80×35×55cm) with test volume 100L. The test medium was aerated non-chlorinated drinking water diluted with deionized water, although no aeration of the solutions once in the test vessels. The flow rate was approximately 21 L/h/test aquarium (≈5-fold volume exchange/day/test vessel). Fish were fed with commercial fish diet (BioMar) at around 1% of the body weight per day in 2 applications per day.

Temperature:	13°C;
pH:	7.9-8.0;
Oxygen content:	9.0 mg/L-9.8 mg/L;
Hardness:	104 mg/L $CaCO_3$;
Total organic carbon:	0.8-1.0 mg/L;
Photoperiod:	16h light: 8h dark;

Analytics: Determination of test substance concentrations in water and fish was conducted by measuring total radioactivity using Liquid Scintillation Counting on days -1, 0, 0.125, 1, 2, 3, 7, 10 and 14. Two 10 ml samples taken from the middle per test vessel were taken, filled with scintillation cocktail and homogenised by shaking, then tested for radioactivity.

Concurrently (except on days -1 and 0), 5 fish were sacrificed from each test vessel, rinsed, blotted dry, weighed and length measured, and dissected into 4. Each part was weighed, dried overnight and combusted, and then promptly analysed by liquid scintillation.

Statistics: BCF values were calculated based on steady state concentration (plateau phase of the uptake period) in fish (BCF_{ss}) and based on the uptake and depuration curves by using a first order (one-compartment) biokinetic model (BCF_k). BCF values were further normalized to 5% fish lipid content by multiplying by 0.05 and divided by a lipid normalisation factor, and growth during the experiment was corrected for by calculating the growth rate constant and taking it away from the depuration rate constant to then divide the uptake rate constant.

II. RESULTS AND DISCUSSION

Validity criteria
The following validity criteria were met (OECD 305 (2012)):

- Temperature variation of less than ±2°C (±0°C)
- Oxygen concentration should be at least ≥60% of saturation (9.0-9.8µg/L)
- Test concentrations ±20% initial mean measured during uptake (minimum 87.0% maximum 100.2%)
- The concentration of the test substance must not be below the solubility of the test substance (test concentration of 10 µg/L for a solubility of 810 µg/L in deionised water)
- ≤10% mortality or other adverse effects in the control and test groups (0%)

Fish status and growth
No mortalities or signs of toxicity were observed. There was no statistically significant difference in fish growth rate between control and treatment group during the experiment, therefore data from both groups were combined to determine the overall growth rate (k_g) for "growth-corrected" calculations. The combined growth rate of both groups (k_g) was 0.0132/day.

Lipid content of fish
The mean lipid content of control fish was 2.3% at the start of exposure, 1.9% at the end of exposure and 3.2% at the end of the test period. The lipid content of the control fish remained constant considering the variability of the individual measurements. Since there was no statistical difference in growth, for calculation of the lipid corrected BCF the mean lipid content in control fish of the uptake period (2.1%) was used.

Analytical results in water and fish tissue
During the uptake period (to day 14) the concentration of the test substance in water remained within ± 20% of the nominal concentration based on radioactivity measurement. Also the additional daily measurements indicated no deviation of >20% from the nominal concentration. The mean concentrations of ^{14}C-BAS 750 F in water during the uptake phase (through day 14) were 9.34 ± 0.43 µg/L (93.4% of the nominal concentration). By day 2 of the depuration period, concentrations were less than 1% of nominal (Table B.9.2.2/3-1).

Table B.9.2.2/3-1: Concentration of BAS 750 F

Sample Day	Mean concentration in whole fish (µg/kg)	Mean concentration in water (µg/L)
Uptake		
-1	-	9.76
0	-	10.02
0.125	398.3	8.7
1	1211	8.94
2	1344.9	9.55
3	1051.9	9.4
7	1474.4	9.27
14	1629.2	9.52
Depuration		
0.125	1399.0	0.43
1	521.2	0.10
2	190.5	0.03
4	80.9	0.004
7	34.4	-

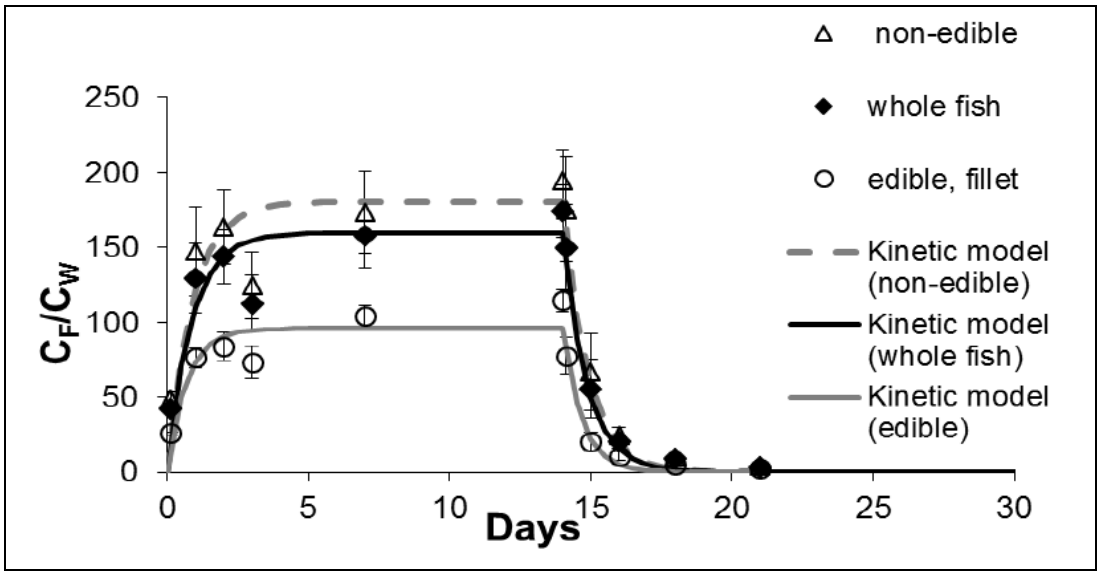
The accumulation of test substance in the edible fish portions was less than in the non-edible portions. The concentration of ^{14}C-BAS 750 F in the whole fish on day 2 was within 20% of the highest measured concentration in fish during the uptake period, indicating that the uptake had become approximately asymptotic with respect to time (see Figure B.9.2.2/3-1), and measurements from days 2-14 were considered for the calculation of the steady state bioconcentration factor (BCF_{ss}). The calculated BCF_{ss} was 147, and 350 when normalized to 5% lipid content.

Bioconcentration kinetics

The measured data from whole fish as well as edible and non-edible portions fit a first order kinetic model allowing an estimation of the uptake and depuration rate constants based on simultaneous curve fitting. Kinetic calculations indicate that concentration in fish reached 95% steady state within 2.6 days.

After the start of depuration, the concentrations in fish progressively declined. After 7 days in clean water the whole body residues in fish had declined to 3% of the mean steady state concentration (CF_{ss}).

Figure B.9.2.2/3/1-1: Uptake and depuration curves as BCF ($C_F(t)/C_W$). Not corrected for growth or lipid content.



The lipid normalized steady state BCF (BCF_{SSL}) values were 350, 224 and 391 for the whole fish, edible and non-edible portions respectively. Based on the kinetic model, the calculated uptake rate constants (k_1) were 187, 140, and 204 day^{-1} for the whole fish, edible and non-edible portions respectively. The calculated growth corrected depuration rate constants (k_{2g}) were 1.16, 1.45, and 1.12 day^{-1} for the whole fish, edible and non-edible portions respectively. The results are summarised in Table B.9.2.2/3-2.

Overall the measured BCF_{ss} values were very similar to the calculated BCF_K values indicating that steady state was reached and that uptake and depuration follow first order kinetics. The most relevant BCF is the growth corrected kinetic BCF normalized to 5% lipid content (BCF_{KLg}) because it incorporates all measurements during uptake and depuration and since it removes the influence of the test fish lipid content. In conclusion the bioconcentration factor BCF_{KLg} was 385 for the whole fish based on total radioactive residues of ^{14}C-BAS 750 F.

Table B.9.2.2/3-2: Uptake and depuration rate constants and bioconcentration factors (BCF) for the whole fish, edible and non-edible portions based on measured and calculated data.

Parameter	Whole fish	Edible	Non-edible
k_g (growth rate constant; day^{-1}) (standard error)	0.0132 (0.0011)	-	-
k_1, (overall uptake rate constant, L/kg/day) (95% confidence interval)	187 (149-225)	140 (113-168)	204 (160-248)
k_2, (overall depuration rate constant, day^{-1}) (95% confidence interval)	1.17 (0.93-1.41)	1.46 (1.17-1.76)	1.13 (0.88-1.38)
k_{2g} (growth-corrected depuration rate constant, day^{-1})	1.16	1.45	1.12
$C_F$ss, (concentration in fish at steady-state, µg/kg) (mean (days 2-14) ± standard deviation)	1375 ± 245	877 ± 176	1533 ± 273
C_W (concentration in the water, µg/L) (mean (days 0 – 14) ±standard deviation)	9.34 ± 0.43	--	--
L_n (lipid normalization factor) (mean during uptake)	0.021	--	--
BCFss (steady-state BCF; L/kg) (mean (days 2-14) ± standard deviation)	147 ± 26	94 ± 19	164 ± 29
BCF_{SSL} (lipid normalized steady-state BCF; L/kg)	350	224	391
BCF_K (kinetic BCF; L/kg)	160	96	181
BCF_{Kg} (growth-corrected kinetic BCF; L/kg)	162	97	183
BCF_{KLg} (lipid-normalized kinetic BCF_{Kg}; L/kg) [a]	**385**	–	–
$t_{1/2}$, (depuration half-life; day)	0.59	0.47	0.61
$t_{1/2g}$ (growth-corrected half-life, day)	0.60	0.48	0.62
Time to 95% steady state (growth-corrected, day)	2.6	2.1	2.7

[a] The most relevant BCF in this study is the growth corrected kinetic BCF normalized to 5% lipid content, BCF_{KLG}.

III. CONCLUSION

In a flow-through bioconcentration study, rainbow trout were exposed to BAS 750 F at 0.010 mg/L (nominal) in water for an uptake period of 14 days. 95% steady state was reached within 2.6 days. The most relevant BCF is the growth corrected kinetic BCF normalized to 5% lipid

content (BCF_{KLg}) for the whole fish which was determined to be 385 based on total radioactive residues of BAS 750 F.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed BCF is the BCF_{KLg} of 385. The RMS notes that the usual duration for this test is 28 days. However, as the steady state was reached before 3 days and depuration meant that after 7 days only 3% of the accumulated active remained. Therefore the reduced duration of the experiment is not expected to have adversely affected the results. While only one test concentration has been studied, this is acceptable according to OECD 305 (2012) this is under the proviso that a justification has been submitted by the applicant. The justification submitted by the applicant is presented below: *"The OECD guideline 305 on "Bioaccumulation in Fish" (adopted 2012) states that "the testing of only one test concentration can be considered sufficient, when it is likely that the bioconcentration factor (BCF) is independent of the test concentration". The guidance further mentions that for non-polar organic substances the exposure of fish to a single concentration is expected to be sufficient as no concentration effects are expected.*

An extensive data review of Creton et al. (2013) supports the use of only one test concentration in BCF studies specifically for plant protection products. The researchers reviewed 55 active substances with a wide log K_{OW} range (-0.81 to 6.9) and various modes of action. They compared BCF values from low and high test concentrations (generally a factor of 10 apart) and found a linear relationship for all examined dimensions (whole body, edible and non-edible tissue). Among the 55 reviewed active substances also triazoles were present, e.g. prothioconazole, triticonazole, metconazole and epoxiconazole. The ratio between the 'low-concentration BCF' and 'high-concentration BCF' for triazoles differed only between 0.85 and 1.19. Paragraph 78 from the OECD 305 guidelines defines that a concentration dependence is not indicated if uptake and depuration rate (and therefore also the kinetic BCF as a function of these rate constants) vary by less than 20% from two test concentrations. This is the case for plant protection products and specifically for triazole fungicides.

The review by Creton et al. (2013) demonstrates clearly that no significant difference between the BCF in low and high concentrations can be found for plant protection products, although the data set considered substances with highly differing physico-chemical properties and even different fish species in the tests.

In order to minimize vertebrate testing and since no concentration effect is expected for the triazole fungicide BAS 750 F, only one test concentration was chosen to be sufficient for the respective bioconcentration study in fish."

References:

OECD (2012): Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing

Creton S., Weltje L., Hobson H., Wheeler J.R. (2013): Reducing the number of fish in bioconcentration studies for plant protection products by reducing the number of test concentrations. Chemosphere 90 (2013), 1300–1304.

The Creton *et al* (2013) paper demonstrated that there were no statistically significant differences between BCF values determined using high or low test concentrations. The RMS notes that only some of the triazole data presented in the paper has been included in the above consideration by the Applicant. Additional triazole data for tetraconaole and tebuconzole is present – these indicate ratios of 1.04 and 1.69, respectively. It is noted that the ratio for tebuconazole is the second highest ratio reported for all active substances included in the study – and appears to be somewhat higher than that reported for the other 5 triazole fungicides (prothioconazole, triticonazole, metconazole, tetraconazole and epoxiconazole). Therefore, the range in values for triazole fungicides (0.85-1.69) is somewhat wider than stated above.

The following consideration is provided for illustrative purposes/supporting information only. Given that is unknown whether a high or low concentration was tested for BAS 750 F – the range in ratios indicates that the BCF value could potentially be 15% lower or 70% higher if a further concentration had been tested. It is noted that the BCF value for BAS 750 F is 385. Therefore, assuming a worst

case ratio of 1.7 for triazoles – the BCF value would potentially increase to 654.5. This value is still below the bioaccumulation trigger value of BCF >2000. As a consequence, it is not considered that testing a further concentration would have an impact on this evaluation. In addition, it is noted that the Creton *et al* (2013) study states that "data were available from 166 studies of which 108 used only one test concentration". Therefore, the use of single test concentrations appears to be relatively common.

The agreed endpoint suitable for use in the risk assessment is:
BCF_{KLg} of 385

B.9.2.3. Potential for endocrine disruption

Member states should note that there are currently no defined criteria for identifying endocrine disruptors or interpreting the significance of any effects in ecotoxicology studies under the Commission Regulation (EU) N° 2009/1107. Consequently, endpoints have not been defined and a risk assessment has not been conducted, and it cannot be concluded if any endocrine disruptive effects are taking place or not.

Report: B.9.2.3/1
[redacted] 2015b
BAS 750 F Fish sexual development test on the zebrafish (*Danio rerio*)
2015/1099093
Guidelines: OECD 234
GLP: Yes

Report: B.9.2.3/1a
Obermann M., 2014a
Concentration control analysis of BAS 750 F, Reg.No. 5834378 in mixing-water, GV/T Project No. 56F0741/11E177
2014/1161851

Report: B.9.2.3/1b
Obermann M., 2015a
Report Amendment No. 1-Concentration control analysis of BAS 750 F, Reg.No. 5834378 in mixing-water, GV/T Project No. 56F0741/11E177
2015/1117846

Report: B.9.2.3/1c
Obermann M., 2015b
Report amendment no. 2 to final report: Concentration control analysis of BAS 750 F, Reg.No. 5834378 in mixing-water, GV/T Project No. 56F0741/11E177
2015/1181295

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD 001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Zebrafish (*Danio rerio*) eggs less than 6 hours old, sourced from in-house rearing from 4 spawning pairs.

Test design:	Flow through system (69 d) of 3 test substance concentrations plus a dilution water control, 4 replicates per treatment with 30 fertilised eggs per replicate. Eggs and larvae were exposed in egg cups and juveniles were transferred to stainless steel aquaria at a suitable size for the remainder of exposure. The test solution flowed continuously from the mixing tank through a flow splitter which split the test water into 4 equal parts for the 4 replicate test vessels. On day 68/69 fish were sacrificed (workload was too high to sacrifice all fish on the same day, therefore A and B replicates were sacrificed on day 68, C and replicates were sacrificed on day 69) and the body length and weight of surviving individuals were determined. Daily assessment of survival and signs of toxicity or abnormal behaviour. Histological evaluation of gonad development and sex ratio after sacrifice. Determination of vitellogenin(VTG) in head and tail of all fish at the end of exposure. VTG content was determined with a commercially available ELISA kit (zebrafish vitellogenin, Biosense laboratory, Bergen, Norway) and was calculated per mg whole fish body.
Endpoints:	NOEC values based on hatching success, survival, toxic signs, body weight, body length, maturity, sex ratio, gonad histopathology and vitellogenin.
Test concentrations:	Due to low solubility of the test substance, test concentrations were dilutions of a saturated solution of BAS 750 F. Consequently it was not possible to set nominal concentrations, but instead represent the results as percentages of the saturated solution. The test solutions were a control (dilution water), 0.010, 0.021 and 0.041 mg BAS 750 F/L (nominal), corresponding to mean measured concentrations of 0.010, 0.022 and 0.045 mg a.s./L. Homogeneity of the test solutions was demonstrated by filtration and <20% difference in comparison to unfiltered stock.
Preparation:	Saturated stock solution using saturation columns prepared by adding 3.5g of test substance to approximately 200 ml of acetone, each used for up to 14 days (generally two in parallel with a new column prepared once per week). Throughout the exposure period the stock solution was continuously diluted with aerated dilution water at a constant rate, and the mixture flowed into four test vessels via a flow splitter.
Test conditions:	Test vessels were stainless steel aquaria (38.5×23.5×29.0cm) with a test volume of 24L. The egg cups were cylindrical glass vessels, diameter 19cm (water volume: 1.7 L). Dilution water was non-chlorinated drinking water (diluted with deionized water). Feeding was *ad libitum* with live brine shrimp nauplii (*Artemia* sp.) and commercial fish diet ("Tetramin" and/or "Sera Micron") from day 6 onwards. There was slight aeration from day 34 onwards in response to low dissolved oxygen (68%). Temperature: 25.4 – 26.9°C; pH: 7.9-8.1; Oxygen content: 5.6 mg/L-8.7 mg/L; Water hardness: 1.04-1.13mmol/L; Conductivity: 275-282µS/cm; Acid capacity: 2.48 – 2.52mmol/L; Light intensity: 91-170lux; Photoperiod: 16 hours light: 8 hours dark;

	Flow rate:	5 L/hour/test vessel and approximately a 5 fold exchange every 24h.
Analytics:		Analytical verification of test substance concentrations was conducted using an HPLC-method with MS detection. The limit of quantification was 0.001 mg/L. Samples were collected from one replicate per test group on day -6 to confirm that concentrations were within ±20% of the nominal concentration. Further samples were collected from alternating replicates on days 0 7, 14, 21, 28, 35, 42, 49, 56, 63 and 68. Samples from all test groups were taken from the middle of the test vessel.
Statistics:		One-sided Jonckheere-Terpstra test or one-sided Wilcoxon-test for survival, one-sided William´s test or one-sided Dunnett's test for weight and length data, one-sided William's test or one-sided Dunnett's test for maturity index and two-sided Jonckheere-Terpstra test or two-sided Wilcoxon test for sex ratio.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 234 (2011) :

- ≥60% dissolved oxygen (minimum 68%)
- Temperature within ±1.5°C of the mean (25.4-26.9°C)
- Maintain concentrations within ±20% of mean measured (84.4-117.2%)
- ≥80% hatching success (98%)
- ≥70% post-hatch survival (100%)

Analytical verification of test substance concentration was conducted in all test substance concentrations at test initiation, at weekly intervals until day 63 and at test termination (day 68). Mean measured concentrations of BAS 750 F ranged from 82.0% to 120.0% of nominal over the exposure period. The following biological results are based on mean measured concentrations.

Table B.9.2.3/1-1: Analytically measured test concentrations

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L)	% of nominal concentration
0.01	0.0095	97.2
0.021	0.0216	102.4
0.041	0.0423	110.3

Hatching started simultaneously in all test groups on day 3 and was complete by day 5. No test substance-related effect was observed on the time to start or end of hatching. Hatching success ranged from 93-100% for the replicates of the control group and there was no statistically significant decrease in hatching success in any of the treatment groups in comparison to the control group. Survival from hatch to the end of swim-up was 100% for all replicates of the control group and there was no statistically significant decrease in survival in any of the treatment groups in comparison to the control group. In all test groups, larval swim-up started on day 5 of exposure and was complete simultaneously with the control on day 6. From the end of swim-up to the end of exposure (day 6-68/69) survival was 93-100% for the replicates of the control group. The survival from the end of swim-up to the end of exposure (day 6-68/69) as well as the overall survival (day 0-68/69) was not statistically decreased in any treatment group in comparison to the control group. There was no statistically significant test substance-related effect on survival in any test concentration.

There were no signs of toxicity or abnormalities observed among the replicates of the control group and the treatment groups with two exceptions in the treatment group of 0.021 mg a.s./L. In this treatment group two deformed fish were identified at the end of exposure. Due to the low incidence and lack of any observations in the next higher treatment group (0.041 mg a.s./L), these are considered incidental findings and are not test substance related. In comparison to the control group the mean wet weights and length of the surviving male and female fish at the end of the exposure period were not statistically significantly decreased in the treatment groups.

There was a mean of 50% male fish in the control and means of 50-56% males in the treatment groups. There was no statistically significant difference in sex ratio between the control and treatment groups. There were also no significant differences between controls and treatments in maturity index or biomarker vitellogenin concentration in head/tail homogenates. The results are summarised in Table B.9.2.3/1.

Table B.9.2.3/1-1: Chronic toxicity of BAS 750 F to zebrafish (*Danio rerio*) in a fish sexual development test (69 d)

Concentration (nominal) [mg a.s./L]		**Control**	**0.010**	**0.021**	**0.041**
Concentration (mean measured) [mg a.s./L]		**--**	**0.010**	**0.022**	**0.045**
Mean hatching success [%]		98	97	98	98
Mean survival of larvae from hatch until end of swim-up (day 6) [%]		100	100	100	100
Mean survival of juveniles (day 6 – 68/69) [%]		97	99	97	97
Mean survival from day 0 to test termination (day 68/69) [%]		94	96	95	96
Wet weight (day 68/69) [mg] #	males	561.4 ± 19.9	559.8 ± 25.6	564.4 ± 24.8	556.2 ± 22.9
	females	774.9 ±16.8	751.6 ±57.7	823.4 ±31.8	780.8 ±25.2
Total length (day 68/69) [cm] #	males	3.9 ± 0.02	3.8 ± 0.02	3.8 ± 0.07	3.9 ± 0.06
	females	4.1 ± 0.01	4.0 ± 0.05	4.1 ± 0.02	4.0 ± 0.01
Vitellogenin concentration in fish (day 68/69) [ng/mg] #	males	3.3 ± 1.71	3.8 ± 1.82	2.3 ± 0.64	2.4 ± 0.81
	females	450.6 ± 174.76	536.7 ± 217.53	547.5 ± 27.83	490.7 ± 141.97
Sex ratio	males	57	61	64	58
	females	56	53	50	57
	% males	50	53°	56	50
Maturity index #	males	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
	females	3.01 ± 0.083	3.02 ± 0.092	3.07 ± 0.136	2.94 ± 0.041
		Endpoints [mg BAS 750 F/L] (mean measured)			
$NOEC_{overall}$		≥ 0.045			

Mean value ± standard deviation

° One fish was excluded from statistical evaluation due to lack of gonadal tissue

The NOEC is given as primary endpoint, since no dose-response relationship was derived from the study which could be used for EC_x calculations.

III. CONCLUSION

In a sexual development study with zebrafish (*Danio rerio*) the overall NOEC for BAS 750 F was determined to be ≥ 0.045 mg a.s./L based on mean measured (mm) concentrations.

RMS Comment: The study is considered acceptable. The RMS notes the high percentage coefficients of variation for male vitellogenin measurements. This was caused by some males having abnormally high VTG values. Although the reason is unknown, contamination with female blood has been excluded by only using scalpels once. High VTG individuals were found in the control and all treatment groups independent of concentration. The RMS equally notes that the percentage coefficients of variations were present in the females that also appeared in the control and all treatment groups independent of concentration. No significant differences in VTG concentrations were observed between any concentration and the control for both male and female fish, although no conclusions on endocrine disruption can be drawn based upon this. While the high coefficient of variations may increase the difficulty of detecting statistically significant differences, no clear dose dependent effects on VTG appear to be present for either gender. The method of analysis was confirmed as validated (III CP B.5.1.2).

B.9.2.4. Acute toxicity to aquatic invertebrates

B.9.2.4.1 Acute toxicity to aquatic invertebrates from the active substance

Report:	B.9.2.4.1/1 Brzozowska K., 2014a BAS 750 F (Reg.No. 5834378)-*Daphnia magna*, acute immobilization test 2013/1250866
Guidelines:	OECD 202 (2004), EPA 850.1010
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740; purity: 98.8% analysed.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS) neonates collected from in house culture, aged <24h old at test initiation.

Test design: Static system (48 hours) for 7 test substance concentrations plus control of 4 replicates with 5 daphnids in each. Immobility was assessed after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, and a series of 7 diluted nitrocellulose filtrates (0.45µm filter) of loading 10 mg BAS 750 F/L of nominal concentrations.

Preparation: The 10 mg/L stock was prepared by ultrasonication (10 minutes) and shaking (24h), corresponding to geometric mean measured concentrations of <LoD (limit of detection for the control), 0.156, 0.254, 0.373, 0.591, 0.838,

1.225 and 1.854 mg a.s./L. A reference test with potassium dichromate was performed in January 2014. The 48h EC_{50} was 0.68 mg/L.

Test conditions: Test vessels were 150 ml glass beakers, test volume 100 mL (loading of 20 ml per daphnid) and dilution water "M7" (Elendt medium). No feeding or no aeration was used during the test.

pH	7.37-7.58;
Oxygen concentration:	88%-98%;
Temperature:	20.3-21.7°C;
Photoperiod:	16h light: 8h dark.

Analytics: Analytical verification of test substance concentrations was conducted using a LC-method with DAD detection. The limit of quantification was 0.01 mg/L and the limit of detection was 0.005 mg/L.

Statistics: Probit analysis for determination of the EC_{50} values; Williams Multiple Sequential t-test Procedure (α=0.05) for determination of the NOEC value. ToxRat Professional was used for statistical analysis.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- Control immobilisation ≤10% (0%)
- End of test dissolved oxygen ≥3 mg/L (minimum 88% saturation)

Analytical verification of the test substance concentrations was conducted at the beginning and at the end of the test. The test substance concentrations determined in samples collected at exposure termination were in the range of 85.1% to 109.0% of initial concentrations. This confirms that the test substance concentrations were stable under test conditions and were within ±20% of the nominal concentrations. The following biological results are based on geometric mean measured concentrations due to the poor solubility of BAS 750 F.

After 48h of exposure, no immobility of daphnids was observed in the control and at test substance concentrations of up to and including 0.254 mg a.s./L, whereas 20%, 30%, 45% and 65% immobility were observed at the test substance concentrations of 0.373, 0.591, 0.838 and 1.225 mg a.s./L, respectively. At the highest test substance concentration, 75% of the daphnids were immobile after 48 hours of exposure. Statistically significant effects on mobility of daphnids were detected at the five highest test substance concentrations. For results see Table B.9.2.4.1/1-1.

Table B.9.2.4.1/1-1: Effects of BAS 750 F on *Daphnia magna* mobility

Concentration [mg a.s./L] (geometric mean measured)	**Control**	**0.156**	**0.254**	**0.373**	**0.591**	**0.838**	**1.225**	**1.854**
Immobility (24 h) [%]	0	0	0	5	5	25	20	30
Immobility (48 h) [%]	0	0	0	20 *	30 *	45 *	65 *	75 *
	Endpoints [mg BAS 750 F/L] (geometric mean measured)							
EC_{10} (48 h)	0.338 (95% confidence limits: 0.217-0.441)							
EC_{20} (48 h)	0.481 (95% confidence limits: 0.350-0.597)							
EC_{50} (48 h)	0.944 (95% confidence limits: 0.770-1.213)							
NOEC (48 h)	0.254							

* Statistically significant differences compared to control (Williams Multiple Sequential t-test Procedure, α=0.05); statistically significant differences compared to control were only determined after 48h of exposure.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of BAS 750 F was determined to be 0.944 mg a.s./L based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is EC_{50} 0.944 mg a.s./L. The RMS notes that the temperature differed outside ±1°C (OECD 202 (2004) and the hardness of the test medium is not reported. However as all the validity criteria were met and no negative effects were observed in the control organisms, these deviations are not expected to have adversely affected the experiment. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
EC_{50} 0.944 mg a.s./L (mm)

Report: B.9.2.4.1/2
VanHooser A., 2014a
BAS 750 F: Acute toxicity test with the saltwater mysid, *Americamysis bahia*, determined under flow-through test conditions
2014/7002845
Guidelines: EPA 850.1035
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Americamysis bahia*) juveniles, aged less than 24 hours old and sourced from in-house cultures.

Test design: Flow-through system (96 hours) for 5 test substance concentrations plus a control and a solvent control, with 2 replicates per treatment of 10 mysids

per replicate and 2 retention baskets per aquarium; Mortality and symptoms of toxicity were assessed daily.

Endpoints: LC_{50} (96h), mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.32, 0.63, 1.3, 2.5 and 5.0 mg BAS 750 F/L (nominal). For both controls and nominal concentrations 0.32, 0.63, and 1.3 mg/L the test solutions were clear and colourless throughout the test. At 2.5 mg/L there was a small amount of white precipitate and more white precipitate at 5.0 mg/L throughout the test. The nominal concentrations corresponded to mean measured concentrations (post-centrifugation) of 0.227, 0.415, 0.896, 1.76 and 3.29 mg a.s./L.

Preparation: Three diluter stock solutions were prepared by diluting 5.0607g of the a.s. to a volume of 0.10L with DMF. A Hamilton Model 500 syringe dispenser introduced aliquots of the stock into the diluter system.

Test conditions: Glass aquaria (14 cm×23 cm×16.5cm) with test volume approximately 4L. Mysids were maintained in retention baskets to facilitate observations. The retention baskets were glass petri dish bases (approximately 1.5 cm×10cm) with a stainless steel screen collar (stainless steel mesh, approximate mesh opening 381 µm). The dilution water was artificial saltwater (commercial sea salt mix added to demineralised fresh water), aerated, filtered and sterilized. Live brine shrimp nauplii (*Artemia* sp.) were provided as food at least once daily *ad libitum*.

Flow rate: approximately 12 volume additions per 24 hours;
Salinity: 19.9-20.1‰;
Temperature: 24.0-24.6°C;
pH 7.9-8.1;
Oxygen content: 5.2-7.4 mg/L;
Photoperiod 14h light: 10h dark;
Light intensity: 793lux;

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with MS/MS-detection. 0h and 96h samples were centrifuged for 5 minutes at 3200 rpm to remove any organic or particulate matter. The minimum quantification limit was 0.00250 mg/L.

Statistics: Probit analysis, Trimmed Spearman Karber or Spearman-Karber method for calculation of the LC_{50}. SAS version 9.3 was used for statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria of 850.1035 were met; mortality was <10% in the control organisms (0%). Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically determined concentrations of BAS 750 F ranged from 67% to 77% of nominal concentrations at test initiation and from 62% to 68% of nominal at test termination. The following biological results are based on mean measured concentrations.

After 48 hours of exposure no mortality and no other toxic effects were observed in the control, the solvent control and at test substance concentrations of up to and including 0.896 mg a.s./L, whereas 70% mortality and sub-lethal effects (lethargy) were observed at the second highest test substance

concentration of 1.76 mg a.s./L. At the highest test substance concentration of 3.29 mg a.s./L, 100% mortality occurred. The results are summarised in Table B.9.2.4.1/2-1.

Table B.9.2.4.1/2-1: Acute toxicity (96h) of BAS 750 F to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.32**	**0.63**	**1.3**	**2.5**	**5.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.227**	**0.415**	**0.896**	**1.76**	**3.29**
Mortality [%] (24 h)	0	0	0	0	0	0	100
Symptoms after 24h [#]	none	none	none	none	none	8/20 L	n.d.
Mortality [%] (48 h)	0	0	0	0	0	70	100
Symptoms after 48h [#]	none	none	none	none	none	6/6 L	n.d.
Mortality [%] (72 h)	0	0	0	0	0	95	100
Symptoms after 72h [#]	none	none	none	none	3/20 L	1/1 L	n.d.
Mortality [%] (96 h)	0	0	0	0	0	95	100
Symptoms after 96h [#]	none	none	none	none	3/20 L	1/1 L	n.d.
Endpoints [mg BAS 750 F/L] (mean measured)							
LC_{50} (24 h)	2.41 (95% confidence limits: 1.76-3.29)						
LC_{50} (48 h)	1.53 (95% confidence limits: 1.34-1.74)						
LC_{50} (72 h)	1.30 (95% confidence limits: 1.22-1.38)						
LC_{50} (96 h)	**1.30 (95% confidence limits: 1.22-1.38)**						

[#] L=lethargy
n.d.=not determined; all animals dead

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (96 h) for BAS 750 F was determined to be 1.30 mg a.s./L.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is 96h LC_{50} of 1.30 mg a.s./L. The RMS notes that a white precipitate was observed in some test solutions, and consequently the results may underestimate the toxicity of the active. Given that the sample solutions were centrifuged before analysis, it can be expected that the mean measured samples are representative of the concentrations the test organisms were exposed to, so no further consideration is required. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
48h and 96h LC_{50} of 1.53 and 1.30 mg a.s./L (mm) respectively

Report: B.9.2.4.1/3
VanHooser A., 2015b
BAS 750 F: Effect on new shell growth of the eastern oyster (*Crassostrea virginica*)
2015/7000021
Guidelines: EPA 850.1025, EPA 72-3(e)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance:	BAS 750 F, white solid,
Lot no.	COD-001740
Purity:	98.8% (tolerance ± 1%).

B. STUDY DESIGN

Test species: Eastern oyster (*Crassostrea virginica*) of mean valve height: 35.4±1.9mm (range 32.4-39.3mm), sourced from "Circle C Oyster Ranch",Ridge, Maryland, USA. Test organisms were acclimatised for three days prior to test initiation.

Test design: Flow-through system (96 hours); 6 test substance concentrations plus a control and a solvent control, 2 replicates for each test substance concentration and the controls with 10 oysters per replicate (20 animals per treatment); daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.

Endpoints: LC_{50}, EC_{50} and NOEC for mortality, shell growth inhibition and symptoms of toxicity such as slow valve closure or lack of feeding activity.

Test concentrations: Control (dilution water), solvent control (0.050 mL dimethylformamide/L), 0.14, 0.25, 0.45, 0.80, 1.4 and 2.6 mg BAS 750 F/L (nominal), corresponding to mean measured concentrations of 0.111, 0.174, 0.335, 0.623, 1.12 and 1.80 mg a.s./L. All test solutions appeared clear and colourless throughout.

Preparation: Diluter stock solutions were prepared by adding 10.5263g of the a.s. to a volume of 0.20L with DMF. A Hamilton Model 500 syringe dispenser introduced aliquots of the stock into the diluter system.

Test conditions: Glass aquaria (21.5×37.0 ×18.0cm), test volume approximately 8.4L; artificial seawater, filtered, aerated and sterilized; food marine microalgal concentrate

Flow rate: approximately 12.9 volume additions per 24 hours in each test vessel;

Salinity:	19.4-19.5‰;
Temperature:	19.1-20.6°C;
pH:	7.9-8.5;
Oxygen content:	5.0 mg/L-7.7 mg/L;
Photoperiod:	16h light: 8h dark;
Light intensity:	420-541lux.

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with MS/MS-detection at 0 and 96 h. Samples were centrifuged for 10 minutes at 3200rpm prior to analysis. The minimum quantification limit was 0.00250 mg a.s./L.

Statistics: Four-parameter logistic (sigmoid-shaped) model for calculation of EC_{50}, one tailed Dunnett's Test for determination of the NOEC value for shell deposition data ($p<0.05$). SAS software (version 9.3) was used for statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met:

- ≤10% control mortality (0%)
- ≥60% dissolved oxygen (minimum 63%)
- ≥2mm control shell growth (3.3mm)
- No evidence of spawning

Analytical verification of test substance concentrations was conducted in each concentration at test initiation and at test termination. Mean measured concentrations for BAS 750 F ranged from 65% to 78% of nominal concentrations at test initiation and from 71% to 87% of nominal at test termination. The following biological results are based on mean measured concentrations.

There was a noticeable reduction in faecal material observed at the test substance concentrations of 1.12 mg a.s./L and 1.80 mg a.s./L as compared to the control throughout the test. After 96 hours of exposure, no mortality of oysters occurred in the control and the solvent control and at test substance concentrations of up to and including 1.12 mg BAS 750 F/L, whereas 20% mortality was observed at the highest test substance concentration of 1.80 mg a.s./L. Shell growth was statistically significantly inhibited at the three highest test substance concentrations compared to the control (Dunnett's Test, $p<0.05$). The results are summarised in Table B.9.2.4.1/3-1.

Table B.9.2.4.1/3-1: Effects on shell growth (96 h) of BAS 750 F to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.14**	**0.25**	**0.45**	**0.80**	**1.4**	**2.6**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.111**	**0.174**	**0.335**	**0.623**	**1.12**	**1.80**
Mortality after 96h [%]	0	0	0	0	0	0	0	20
Inhibition of shell growth after 96h [% difference from control]	--	-7	1	-6	8	27 *	60 *	92 *
	Endpoints [mg BAS 750 F/L] (mean measured)							
LC_{50} (96 h)	> 1.80							
EC_{50} (96 h)	0.947 (95% confidence limits: 0.856-1.04)							
NOEC (96 h)	0.335							

* Statistically significant difference compared to the control (Dunnett's Test, $p<0.05$).

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the LC_{50} (96 h) for BAS 750 F was >1.80 mg a.s./L and the EC_{50} (96 h) was 0.947 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.335 mg a.s./L (mean measured).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an EC_{50} of 0.947 mg a.s./L. The method of analysis was confirmed as validated

(III CP B.5.1.2).

B.9.2.4.2 Toxicity to aquatic invertebrates from metabolites

Report:	B.9.2.4.2/1 Backfisch K.,Härthe N., 2015a Acute toxicity of Reg.No. 6003432 (M750F007; metabolite of BAS 750 F) to *Daphnia magna* STRAUS in a 48 hour static test 2015/1003915
Guidelines:	OECD 202, EPA 850.1010 draft April 1996
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F007 (metabolite of BAS 750 F), batch no. L87-32-1; purity: 97%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates, in house culture, between 2 and 24 hours old.

Test design: Static system (48 hours) for 5 test substance concentrations plus control, 4 replicates with 5 daphnids each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, 0.625, 1.25, 2.5, 5.0 and 10.0 mg/L (nominal). A reference test with potassium dichromate was performed the same year of the study. The EC_{50} of 1.37 mg/L was within the expected range.

Preparation: Serial dilution of s stock solution prepared from adding 11.91 mg of the test substance to 1155mL of M4 water. The stock was the sonicated for 30 seconds, and the solution was clear.

Test conditions: Glass beakers, test volume 50 mL, dilution water "M4" (Elendt medium); no feeding, no aeration.

pH:	7.92-8.6;
Oxygen content:	8.23-8.53 mg/L;
Temperature:	21.4-22.2°C;
Total hardness:	2.47mmol/L,
Conductivity:	689µS/cm (at test initiation)
Photoperiod:	16h light: 8h dark;
Light intensity:	490-550lux;

Analytics: Analytical verification of test substance concentrations was conducted using an HPLC-method with MS detection. The limit of quantification was 0.001 mg/L and the limit of detection was 0.00025 mg/L

Statistics: No analysis required.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- Control immobilisation ≤10% (0%)
- End of test dissolved oxygen ≥3 mg/L (minimum 8.23 mg/L)

Analytical verification of the test substance concentrations was determined at the beginning and at the end of the test. The mean measured values were in range of 78.9 to 100% at test initiation and 76.7 to 98.2% at test termination. The following biological results are based on nominal concentrations.

No significant effects on the mobility of *Daphnia magna* could be observed at all concentrations after 24 and 48 h. For results see Table B.9.2.4.2/1-1.

Table B.9.2.4.2/1-1: Effects of M750F007on *Daphnia magna* mobility

Concentration (nominal)	**Control**	**0.625**	**1.25**	**2.5**	**5.0**	**10.0**
Immobility (24 h) [%]	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	0	0	0
	Endpoints [mg M750F007/L] (nominal)					
EC_{50} (48 h)	> 10					
NOEC (48 h)	≥ 10					

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} and the NOEC of the metabolite M750F007 were determined to be >10.0 mg/L and ≥10 mg/L respectively based on nominal concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is EC_{50} >10 mg/L. The RMS notes that the although mean measured concentrations were not maintained within ±20% of the nominal, for 10 mg/L the recovery was 100% at initiation and 98.2% at 48h. As the endpoints are >10 mg/L, basing the endpoint off nominal concentrations is acceptable. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
EC_{50} >10 mg/L (mm)

Report: B.9.2.4.2/2
Rzodeczko H., 2015c
Reg.No. 5863469 (metabolite of BAS 750 F, M750F006)-*Daphnia magna*, acute immobilization test
2015/1001492
Guidelines: OECD 202 (2004), EPA 850.1010
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F006 (metabolite of BAS 750 F), batch no. L87-30; purity: 98.9%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS) neonates collected from in house culture aged <24h old at test initiation.

Test design: Static system (48 hours) for 5 test substance concentrations plus control and solvent control (N,N-dimethylformamide (DMF)); 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, solvent control (DMF), 16-fold dilution, 8-fold dilution, 4-fold dilution and 2-fold dilution of the filtrate and the filtrate itself of a loading of 10 mg M750F006/L. These corresponded to geometric mean measured concentrations of 0.47, 0.84, 1.83, 3.68 and 7.67 mg/L. All filtrates were visually homogenous and transparent without any undissolved particles. A reference test with potassium dichromate was performed the same year of the study. The 48h EC_{50} of 0.66 mg/L was within the expected range.

Preparation: 83.2 mg of test substance were added to 830µL of DMF. 200µL of this solution was mixed with Elendt M7 Medium up to a volume of 2L. This solution was then sonicated for 15 minutes and filtered through a 0.45µm nitrocellulose membrane.

Test conditions: Test vessels were 150 mL glass beakers and lids, test volume 100 mL, dilution water "M7" (Elendt medium). No feeding or no aeration was performed during the study.

pH:	7.41-7.53;
Dissolved oxygen:	91-94%;
Temperature:	20.7-21.6°C;
Total hardness:	188.0-196.4 mg $CaCO_3$/L;
Conductivity:	548-555µS/cm;
Photoperiod:	16h light: 8h dark;

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD detection. The limit of quantification was 0.001 mg/L and the limit of detection was 0.005 mg/L.

Statistics: Probit analysis for calculation of EC_{50}; Fisher's Exact Binominal Test with Bonferroni Correction for determination of the NOEC (α=0.05). ToxRat Professional 2.10 was the software used for statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- Control immobilisation ≤10% (0%)
- End of test dissolved oxygen ≥3 mg/L (minimum 91%)

Analytical verification of test substance concentrations was conducted in each test substance concentration at the beginning of the test and at the end of the test. The geometric mean concentrations of the test substance were 7.67, 3.68, 1.83, 0.84, 0.47 mg/L corresponding to the 10 mg/L filtrate and

the 2, 4, 8 and 16-fold dilutions respectively. The concentrations of test substance determined in samples collected at exposure termination were in the range of 85.68% to 111.71% of initial concentrations. The following biological results are based on geometric mean measured concentrations.

After 48 hours of exposure, no immobility of daphnids was observed in the control and at the lowest test substance concentration of 0.47 mg M750F006/L, whereas 15%, 20%, 55% and 60% immobility occurred at the test substance concentrations of 0.84, 1.83, 3.68 and 7.67 mg/L, respectively. Statistically significant effects on mobility of daphnids after 48 hours of exposure were observed at the two highest tested concentrations of 3.68 and 7.67 mg/L. No other sub-lethal effects were observed during the test. For results see Table B.9.2.4.2/2-1.

Table B.9.2.4.2/2-1: Effects of M750F006 (metabolite of BAS 750 F) on *Daphnia magna* mobility

Concentration [mg/L] (geometric mean measured)	**Control**	**Solvent Control**	**0.47**	**0.84**	**1.83**	**3.68**	**7.67**
Immobility (24 h) [%]	0	0	0	0	0	35	55
Immobility (48 h) [%]	0	0	0	15	20	55 *	60 *
	Endpoints [mg M750F006/L] (geometric mean measured)						
EC_{50} (48 h)	4.42 (95% confidence limits: 3.03 – 8.05)						
NOEC (48 h)	0.47						

* Statistically significantly different compared to the control after 48h of exposure (Fisher's Exact Binominal Test with Bonferroni Correction, α=0.05).

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of the metabolite M750F006 was determined to be 4.42 mg/L based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is EC_{50} 4.42 mg/L. As there were mortalities observed in line with the probit curve at 0.84 and 1.83 mg/L, even though they were not significant, treatment related mortality appears to be present at these test concentrations. Therefore the NOEC has been adjusted to reflect this. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
EC_{50} 4.42 mg/L (mm)

Report:	B.9.2.4.2/3 Rzodeczko H., 2015d Reg.No. 6003433 (metabolite of BAS 750 F, M750F005)-*Daphnia magna*, acute immobilization test 2015/1001490
Guidelines:	OECD 202 (2004), EPA 850.1010
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F005 (metabolite of BAS 750 F), batch no. L87-34; purity: 99.4%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; <24h old at test initiation; not first brood progeny.

Test design: Static system (48 hours) for 5 test substance concentrations plus control and solvent control (N,N-dimethylformamide (DMF)) with 4 replicates with 5 daphnids in each. Immobility was assessed after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, solvent control (DMF), serial dilutions of a filtrate of a loading of 10 mg M750F005/L, factor 2 up to 16, corresponding to geometric mean measured concentrations of 0.53, 1.07, 2.13, 4.23 and 8.58 mg/L. All filtrates were visually homogenous and transparent without any undissolved particles. A reference test with potassium dichromate was performed the same year of the study. The 24h EC_{50} of 0.91 mg/L was within the expected range.

Preparation: 80.3 mg of test substance were added to 800µL of DMF. 200µL of this solution was mixed with Elendt M7 Medium up to a volume of 2L. This solution was then sonicated for 15 minutes and filtered through a 0.45µm nitrocellulose membrane.

Test conditions: Test vessels were 150 mL glass beakers and lids, test volume 100 mL, dilution water "M7" (Elendt medium). No feeding or no aeration was performed during the study.

pH:	7.39-7.50;
Oxygen:	88-94%;
Temperature:	20.7-21.6°C;
Total hardness:	198.4-202.8 mg $CaCO_3$/L;
Conductivity:	543-551µS/cm;
Photoperiod:	16h light: 8h dark;

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD detection. The limit of quantification was 0.005 mg./L and the limit of detection was 0.001 mg/L.

Statistics: No analysis required.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- Control immobilisation ≤10% (0%)
- End of test dissolved oxygen ≥3 mg/L (minimum 88%)

Analytical verification of test substance concentrations was conducted in each test substance concentration at the beginning of the test and at the end of the test. The geometric mean concentrations

of the test substance were 8.58, 4.23, 2.13, 1.07 and 0.53 mg/L corresponding to the 10 mg/L loading and the 2, 4, 8 and 16-fold dilutions respectively. The concentrations of test substance determined in samples collected at exposure termination were in the range of 97.59% to 100.55% of initial concentrations. The following biological results are based on geometric mean measured concentrations.

After 48 hours of exposure, no immobility of daphnids was observed in the control and all test substance concentrations. Consequently the EC_{50} value was >8.58 mg/L and the NOEC was 8.58≥mg/L.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of the metabolite M750F005 was determined to be >8.58 mg/L based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is EC_{50} >8.58 mg/L. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
EC_{50} >8.58 mg/L (mm)

Report:	B.9.2.4.2/4 Rzodeczko H., 2015e Reg.No. 6010286 (metabolite of BAS 750 F, M750F008) - *Daphnia magna*, acute immobilization test 2015/1001493
Guidelines:	OECD 202 (2004), EPA 850.1010
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F008 (metabolite of BAS 750 F), batch no. L85-94; purity: 96.5%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; <24h old at test initiation and not first brood progeny.

Test design: Static system (48 hours), 5 test substance concentrations plus control and solvent control (N,N-dimethylformamide (DMF)); 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, solvent control (DMF), serial dilutions of a filtrate of a loading of 10 mg M750F008/L, factor 2 up to 16, corresponding to geometric mean measured concentrations of 0.47, 0.97, 1.90, 3.90 and 8.07 mg/L. All filtrates were visually homogenous and transparent without any undissolved particles. A reference test with potassium dichromate was performed

regularly. The previous reference 24h EC_{50} of 0.91 mg/L was within the expected range.

Preparation: 249.8 mg of test substance were added to 250µL of DMF. 20µL of this solution was mixed with Elendt M7 Medium up to a volume of 2L. This solution was then sonicated for 15 minutes and filtered through a 0.45µm nitrocellulose membrane.

Test conditions: Test vessels were 150 mL glass beakers and lids, test volume 100 mL, dilution water "M7" (Elendt medium). No feeding or no aeration was performed during the study.

pH	7.37-7.53;
Oxygen:	89%-96%;
Temperature:	19.3-20.2°C;
Total hardness:	196.0-199.8 mg $CaCO_3$/L;
Conductivity:	539-549µS/cm;
Photoperiod:	16h light: 8h dark;

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD detection. The limit of quantification was 0.0005 mg/L and the limit of detection was 0.0001 mg/L.

Statistics: No analysis required.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- Control immobilisation ≤10% (0%)
- End of test dissolved oxygen ≥3 mg/L (minimum 89%)

Analytical verification of test substance concentrations was conducted in each test substance concentration at the beginning of the test and at the end of the test. The geometric mean concentrations of the test substance were 8.07, 3.90, 1.90, 0.97, 0.47 mg/L corresponding to the 10 mg/L loading and the 2, 4, 8 and 16-fold dilutions respectively. The concentrations of test substance determined in samples collected at exposure termination were in the range of 97.79% to 108.89% of initial concentrations. Biological results are based on geometric mean measured concentrations.

After 48 hours of exposure, no immobility of daphnids was observed in the control and all test substance concentrations. Consequently the EC_{50} value was >8.07 mg/L and the NOEC was ≥8.07 mg/L.

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of the metabolite M750F008 was determined to be >8.07 mg/L based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is EC_{50} >8.07 mg /L and a NOEC of ≥8.07 mg/L. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
EC_{50} >8.07 mg/L (mm)

Report:	B.9.2.4.2/5 Haerthe N., 2016 Acute toxicity of Reg. No. 5924326 (M750F003; metabolite of BAS 750 F) to *Daphnia magna* Straus in a 48 hour static test 2016/1289876
Guidelines:	OECD 202 (2004), EPA 850.1010
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F003 (metabolite of BAS 750 F), batch no. L84-250; purity: 99.6%.

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture; <24h old at test initiation and not first brood progeny.

Test design: Static system (48 hours), 5 test substance concentrations plus control; 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control of M4 water, serial dilutions of a stock solution of 100 mg M750F003/L, corresponding to nominal concentrations of 6.25, 12.5, 25, 50, 100 mg/L. The stock solution was a visibly clear solution. A reference test with potassium dichromate was performed in June 2016 and the 24h EC_{50} of 1.10 mg/L was within the expected range.

Preparation: 99.88 mg of test substance were added to 998.8mL of M4 water. This solution was then stirred and sonificated for around 1 minute, and was clear.

Test conditions: Glass vessels with test volume 50 mL, the dilution water was M4 medium. No feeding or no aeration was performed during the study.

pH	7.97-8.07;
Oxygen:	8.26-8.67 mg/L;
Temperature:	20.6-21.4°C;
Total hardness:	2.57 mmol $CaCO_3$/L;
Conductivity:	689µS/cm;
Light intensity:	490-520lux
Photoperiod:	16h light: 8h dark;

Analytics: Analytical verification of test substance concentrations was conducted using a HPLC-method with MS-detection and external calibration. The limit of quantification was 0.001 mg/L and the limit of detection was 0.0002 mg/L.

Statistics: Determination of the EC_{50} and NOEC was done by Probit analysis using linear maximum Likelihood regression and Fisher's Exact Binomial test with Bonferroni Correction ($p<0.05$). Calculations were performed with ToxRatPro Version 2.10.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 202 (2004)) were met:

- Control immobilisation ≤10% (0%)
- End of test dissolved oxygen ≥3 mg/L (minimum 8.26 mg/L)

Analytical verification of test substance concentrations was conducted in each test substance concentration at the beginning of the test and at the end of the test. The concentrations of test substance determined in samples collected ranged between 103-113% at test initiation and between 104-115% at test termination. As concentrations were maintained within ±20% of the nominal concentrations, the results are based upon nominal concentrations.

After 48 hours of exposure, no statistically significant effects on mobility of the test organisms was observed in the control and all test substance concentrations. Consequently the EC_{50} value was >100 mg/L.

Table B.9.2.4.2/3-1: Effects of M750F003 (metabolite of BAS 750 F) on *Daphnia magna* mobility

Concentration [mg/L] (geometric mean measured)	**Control**	**6.67**	**13.6**	**28.4**	**53.5**	**103.5**
Immobility (24 h) [%]	0	0	0	5	0	0
Immobility (48 h) [%]	0	5	0	15	15	20
Endpoints [mg M750F003/L] (geometric mean measured)						
EC_{50} (48 h)	>103.5					
NOEC (48 h)	13.6					

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of the metabolite M750F003 was determined to be >103.5 mg/L based on nominal concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is EC_{50} >103.5 mg/L. Due to apparent effects being observed at concentrations that appear to be dose related the NOEC is set at 13.6 mg/L despite no significant effects being observed at this concentration. The RMS notes that the hardness of 2.57mmol/L exceeds the recommended maximum of 2.5mmol/L recommended in OECD 203 (2004). Given all the validity criteria were met and no adverse effects were observed in the controls, this deviation is considered acceptable. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
EC_{50} >103.5 mg/L (mm)

B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates

Report: B.9.2.5/1
Janson G-M., 2014a
Chronic toxicity of the BAS 750 F (Reg.No.5834378) to *Daphnia magna* Straus in a 21 day semi-static test
2014/1098028
Guidelines: OECD 211, EPA 850.1300
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8% (tolerance ± 1%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS, clone 5) neonates of >2h and <24 hours old at test initiation and not first brood progeny, from in-house culture (originally obtained from Institute National de Recherche Chimique Appliquee, France).

Test design: Semi-static system (21 days) for 5 test concentrations plus control and solvent control with 10 replicates per treatment and one animal per test vessel. Parent mortality and reproduction was assessed daily (except day 3 and 4) while body length and dry weight was determined at test termination.

Endpoints: EC_x; NOEC, parent mortality, reproduction, growth (parent length and dry weight) and population growth rate.

Test concentrations: Control (dilution water), solvent control (dimethylformamide), 0.005, 0.010, 0.020, 0.040 and 0.080 mg BAS 750 F/L (nominal); corresponding to time-weighted mean measured concentrations of 0.0045, 0.0091, 0.0184, 0.0378 and 0.0773 mg a.s./L, respectively. Stock solutions appeared clear and colourless.

Preparation: Stock solution A was prepared from 8 mg a.s. and 1mL DMF. An additional stock, B, was created by diluting 0.1mL of stock A with M4 water 1000 ml. Test concentrations were created from appropriate amounts of these stock solutions diluted with test medium.

Test conditions: Glass vessels with a test volume of 50 mL of dilution water "M4" (Elendt medium). Feeding occurred daily (except day 3 and 4) with algae (*Desmodesmus subspicatus*). No aeration was performed.

Temperature:	20.7-22.2°C
pH:	7.69-8.26;
Oxygen content:	8.08-8.97 mg/L;
Total hardness:	2.35-2.47 mg/L;
Alkalinity:	0.83-0.87mmol/L;
Conductivity:	621-642µS/cm;
Photoperiod:	16 hours light: 8 hours dark;
Light intensity:	467-970lux;

Analytics:	Analytical verification of test substance concentrations was conducted using an HPLC-method with MS-detection. The limit of quantification was 0.001 mg/L.
Statistics:	Calculation of EC_x using probit-analysis; ANOVA followed by Dunnett's test for determination of the NOEC values ($p<0.05$). ToxRatPro version 2.10 was used to perform the statistical analysis.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 211(2012)) were met:

- ≤20% control parent animal mortality (0%)
- ≥60 mean living offspring per control parent (mean 182 offspring per parent)

Analytical verification of the active substance concentrations was conducted in all treatments at day 0, 2, 9, 12, 14, 16, 19 and 21. Recoveries of BAS 750 F in fresh solutions (measured on days 0, 9, 14 and 19) were in the range of 91% to 101% of nominal concentrations. Measured concentrations of the test substance in old solutions (measured on days 2, 12, 16 and 21) ranged from 81% to 97%. The following biological results are based on nominal concentrations and additionally on time-weighted mean measured concentrations.

Statistical analyses in this study were performed by comparing the pooled control data to the treatment data for the respective parameters. After 21 days of exposure, no parent mortality occurred in the control groups and at the test substance concentrations of up to and including the highest concentration tested. A statistically significant decrease in the number of offspring per parent were observed at the three highest test substance concentrations. The intrinsic rate of increase and day of first brood were significantly affected at the two highest test substance concentrations. Body length of the parent animals was only significantly affected at the highest test substance concentration. The results are summarised in Table B.9.2.5/1-1.

Table B.9.2.5/1-1: Effects of BAS 750 F on *Daphnia magna* parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**Pooled control**	**0.005**	**0.010**	**0.020**	**0.040**	**0.080**
Concentration [mg a.s./L] (time-weighted mean measured)	--	--	--	**0.0045**	**0.0091**	**0.0184**	**0.0378**	**0.0773**
Parent mortality [%]	0	0	--	0	0	0	0	0
Offspring/parent	182	186	184	193	183	163 *	138 *	121 *
CV of offspring/parent [%]	9.5	8.2	--	5.9	8.0	8.4	14.6	16.2
Inhibition in mean cumulative offspring [% of pooled control]	--	--	--	-5.0	0.7	11.3	25.0	34.6
Day of first brood	7.5	7.7	--	7.7	7.7	7.7	8.3 *	9.4 *
Body weight [mg]	0.881	0.891	--	0.874	0.886	0.874	0.861	0.841
Body length [mm]	4.9	4.9	--	5.0	4.9	4.9	4.8	4.7 *
Intrinsic rate of increase (population growth rate)	0.419	0.427	--	0.427	0.416	0.408	0.371 *	0.350 *
	Endpoints [mg BAS 750 F/L]							
EC_{10} (21 d) [a)]	Nominal: 0.0175 (95% confidence limits: 0.0130-0.0237) **Mean measured: 0.0161 (95% confidence limits: 0.0118-0.0220)**							
$NOEC_{overall}$ (21 d)	Nominal: 0.010 **Mean measured: 0.0091**							

CV=coefficient of variation

* Statistically significant effects compared to the pooled control (ANOVA followed by Dunnett's test; $p<0.05$).

[a)] EC_{10} based on cumulative offspring per female data.

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of BAS 750 F was determined to be 0.0091 mg a.s./L. The EC_{10} value was 0.0161 mg a.s./L, based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are a NOEC of 0.0091 mg a.s./L and an EC_{10} of 0.0161 mg a.s./L. The RMS notes that any tests for significance between the controls was not reported, although there does not appear to be any discrepancies between the solvent control and the water control that would be deemed significant and a pooled control was used for comparison with the test concentrations. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
NOEC of 0.0091 mg a.s./L and an EC_{10} of 0.0161 mg a.s./L (mm)

Report: B.9.2.5/2
Janson G.-M., 2015 b
Chronic toxicity of BAS 750 F (Reg.No. 5834378) to *Daphnia longispina* in a 21 day semi-static test

2015/1003912
Guidelines: OECD 211, EPA 850.1300
GLP: Yes

Report: B.9.2.5/2a
Janson G.-M., 2015 c
Report Amendment No.1-Chronic toxicity of BAS 750 F (Reg.No. 5834378) to *Daphnia longispina* in a 21 day semi-static test
2015/1251197
Guidelines: OECD 211, EPA 850.1300

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8% (± 1.0% tolerance).

B. STUDY DESIGN

Test species: Water flea (*Daphnia longispina*) neonates aged >2h and <24 hours old at test initiation and not first brood progeny, from in-house culture (non-GLP, originally obtained from Molecular Ecology University in Landau, Germany).

Test design: Semi-static system (21 days, renewed on days 2,5,7,9,12,14,16 and 19) for 5 test concentrations plus control and solvent control (0.01 mL DMF/L) with 10 replicates per treatment and one animal per test vessel. Parent mortality and reproduction was assessed daily (except day 3 and 4) and body length and dry weight were determined at test termination.

Endpoints: EC_{10}; NOEC, parent mortality, reproduction, growth (parent length and dry weight) and population growth rate.

Test concentrations: Control (dilution water), solvent control (dimethylformamide), 0.015, 0.0225, 0.0338, 0.0507 and 0.0761 mg BAS 750 F/L (nominal); corresponding to time-weighted mean concentrations of 0.0148, 0.0223, 0.0342, 0.0511 and 0.0775 mg a.s./L, respectively. Stock solutions and test solutions appeared clear and without precipitation.

Preparation: Stock solution A was prepared from 7.61 mg a.s. and 1mL DMF. An additional stock, B, was created by diluting 0.1mL of stock A with M4 water 1000 ml. Test concentrations were created from appropriate amounts of these stock solutions.

Test conditions: Glass vessels of test volume 50 mL with dilution water "M4" (Elendt medium). Test organisms were fed algae (*Desmodesmus subspicatus*) daily except on days 3 and 4. No aeration was performed.

Temperature: 20.9-21.9°C;
pH: 7.90-8.15;
Oxygen content: 8.23 -9.30 mg/L;
Total hardness: 2.39-2.53mmol/L;
Alkalinity: 0.84-0.90mmol/L;
Conductivity: 653-664µS/cm;
Photoperiod: 16 hours light: 8 hours dark;

Light intensity: 320-570lux;

Analytics: Analytical verification of test substance concentrations was conducted using an HPLC-method with MS-detection. The limit of detection was 0.00025 mg/L and the limit of quantification was 0.001 mg/L.

Statistics: EC_{10} was calculated using probit-analysis. ANOVA followed by Dunnett's test for determination of the NOEC values ($p<0.05$) based on reproduction, intrinsic rate of increase, body length and dry weight. Bonferroni U-Test for day of first brood. Fisher´s exact test for mortality. ToxRatPro version 2.10 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 211(2012)) were met:

- ≤20% control parent animal mortality (0%)
- ≥60 mean living offspring per control parent (mean 205 offspring per parent)

Analytical verification of the active substance concentrations was conducted in all treatments at day 0, 2, 9, 12, 14, 16, 19 and 21. Recoveries of BAS 750 F in fresh solutions were in the range of 97% to 108% of nominal concentrations. Measured concentrations of the test substance in old solutions ranged from 95% to 106%. The following biological results are based on nominal concentrations and additionally on time-weighted mean measured concentrations.

After 21 days of exposure, no parent mortality occurred in the control groups and at all tested test substance concentrations. Statistically significant differences in the number of offspring per parent were observed at the two highest test substance concentrations and in the intrinsic rate of increase at the highest test substance concentration. Day of first brood, body length, body weight, mobility and age at first reproduction were not significantly affected up to and including the highest test concentration tested. The results are summarised in Table B.9.2.5/2-1.

Table B.9.2.5/2-1: Effects of BAS 750 F on *Daphnia longispina* parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0150**	**0.0225**	**0.0338**	**0.0507**	**0.0761**
Concentration [mg a.s./L] (time-weighted mean)	--	--	**0.0148**	**0.0223**	**0.0342**	**0.0511**	**0.0775**
Parent mortality [%]	0	0	0	0	0	0	0
Offspring/parent	205	206	201	211	207	192 *	141 *
CV of offspring/parent [%]	2.8	3.7	8.9	2.7	2.2	4.3	10.5
Inhibition in mean cumulative offspring [% of control]	--	--	2.1	-2.9	-1.1	6.4	31.1
Day of first brood	6.7	6.7	6.7	6.7	6.7	6.8	7.0
Body weight [mg]	0.322	0.335	0.321	0.343	0.326	0.324	0.309
Body length [mm]	2.6	2.6	2.6	2.6	2.6	2.5	2.5
Intrinsic rate of increase (population growth rate)	0.439	0.438	0.438	0.443	0.437	0.430	0.401 *
	Endpoints [mg BAS 750 F/L]						
EC_{10} (21 d) [a)]	Nominal: 0.0558 (95% confidence limits: 0.0517-0.0603) **Mean measured: 0.0564 (95% confidence limits: 0.0521-0.0610)**						
$NOEC_{overall}$ (21 d)	Nominal: 0.0338 **Mean measured: 0.0342**						

CV=coefficient of variation
* Statistically significant effects compared to the control (ANOVA followed by Dunnett's test; $p<0.05$).
[a)] EC_{10} based on cumulative offspring per female data.

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia longispina* the NOEC of BAS 750 F was determined to be 0.0342 mg a.s./L. The EC_{10} value was 0.0564 mg a.s./L, based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an EC_{10} of 0.0564 mg a.s./L and a NOEC of 0.0342 mg a.s./L. The RMS notes that any tests for significance between the controls was not reported, although there does not appear to be any discrepancies between the solvent control and the water control that would be deemed significant. The method of analysis was confirmed as fully validated (III CA B.5.1.2.6).

The agreed endpoints suitable for use in the risk assessment are:
EC_{10} of 0.0564 mg a.s./L and a NOEC of 0.0342 mg a.s./L (mm)

Report: B.9.2.5/3
Janson G.-M., 2015a
Chronic toxicity of BAS 750 F (Reg.No. 5834378) to *Daphnia pulex* in a 21 day semi-static test
2015/1003913
Guidelines: EPA 850.1300, OECD 211 (2012)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8% (± 1.0% tolerance).

B. STUDY DESIGN

Test species: Water flea (*Daphnia pulex*), neonates aged >2h and <24 hours old at test initiation and not first brood progeny, from in-house culture (non-GLP) and originally obtained from an ornamental fish shop (Bachflohkrebse, Stuttgart, Germany).

Test design: Semi-static system (21 days and renewed on days 2,5,7,9,12,14,16 and 19) for 5 test concentrations plus control and solvent control (0.01 mL DMF/L) with 10 replicates per treatment and one animal per test vessel. Parent mortality and reproduction assessed daily (except day 3 and 4), and body length and dry weight determined at test termination.

Endpoints: EC_{10}; NOEC, parent mortality, reproduction, growth (parent length and dry weight) and population growth rate.

Test concentrations: Control (dilution water), solvent control (dimethylformamide), 0.0125, 0.0188, 0.0282, 0.0423 and 0.0635 mg BAS 750 F/L (nominal); corresponding to time-weighted mean measured concentrations of 0.0121, 0.0184, 0.0276, 0.0407 and 0.0631 mg a.s./L. The stock solutions appeared clear and no precipitation or undissolved particles were observed.

Preparation: A stock solution, A, was prepared from 6 mg a.s. and 1mL DMF. An additional stock, B, was created by diluting 0.1mL of stock A with M4 water 1000 ml. Test concentrations were created from appropriate amounts of these stock solutions and were clear.

Test conditions: Glass vessels; test volume: 50 mL; dilution water "M4" (Elendt medium); feeding: algae (*Desmodesmus subspicatus*) daily except days 3 and 4; no aeration.

Temperature:	20.3-21.7°C;
pH:	7.90-8.21;
Oxygen content:	8.50 mg/L-9.42 mg/L;
Total hardness:	2.53-2.63mmol/L;
Alkalinity:	0.84-0.86mmol/L;
Conductivity:	602-694µS/cm;
Photoperiod:	16 hours light: 8 hours dark;
Light intensity:	270-550lux;

Analytics: Analytical verification of test substance concentrations was conducted using an HPLC-method with MS-detection. The limit of detection was 0.00025 mg/L and the limit of quantification was 0.001 mg/L.

Statistics: EC_{10} was calculated using probit-analysis; ANOVA followed by Dunnett's test for determination of the NOEC values ($p<0.05$) based on reproduction, intrinsic rate of increase, body length and dry weight. Bonferroni U-Test for day of first brood. Fisher´s exact test for mortality. ToxRatPro Version 2.10 was used to perform statistical analysis.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 211(2012)) were met:

- ≤20% control parent animal mortality (0%)
- ≥60 mean living offspring per control parent (mean 253 offspring per parent)

Analytical verification of the active substance concentrations was conducted in all treatments at day 0, 2, 9, 12, 14, 16, 19 and 21 in alternate fresh and aged solutions respectively. Recoveries of BAS 750 F in fresh solutions were in the range of 92.2% to 104% of nominal concentrations. Measured concentrations of the test substance in old solutions ranged from 85.2% to 106%. The following biological results are based on nominal concentrations as the recoveries were within ±20% of the nominal concentration and additionally on time-weighted mean measured concentrations.

After 21 days of exposure, no parent mortality occurred in the control groups and at the test substance concentrations of up to and including the highest concentration tested. Statistically significant differences in the number of offspring per parent and in the intrinsic rate of increase were observed at the two highest test substance concentrations. Day of first brood, body length, body weight, mobility and day of first brood were not affected up to and including the highest test concentration tested. The results are summarised in Table B.9.2.5/3-1.

Table B.9.2.5/3-1: Effects of BAS 750 F on *Daphnia pulex* parent mortality, reproduction and growth after 21 days of exposure

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0125**	**0.0188**	**0.0282**	**0.0423**	**0.0635**
Concentration [mg a.s./L] (time-weighted mean measured)	--	--	**0.0121**	**0.0184**	**0.0276**	**0.0407**	**0.0631**
Parent mortality [%]	0	0	0	0	0	0	0
Offspring/parent	253	255	259	259	263	234 *	224 *
CV of offspring/parent [%]	3.5	2.7	3.1	3.8	1.9	4.3	11.5
Inhibition in mean cumulative offspring [% of control]	--	--	-2.2	-2.3	-3.8	7.7	11.4
Day of first brood	7.0	7.0	7.0	7.0	7.0	7.0	7.3
Body weight [mg]	0.217	0.212	0.200	0.199	0.191	0.200	0.184
Body length [mm]	2.6	2.6	2.6	2.6	2.5	2.5	2.5
Intrinsic rate of increase (population growth rate)	0.470	0.469	0.475	0.474	0.475	0.455 *	0.439 *
	Endpoints [mg BAS 750 F/L]						
EC_{10} (21 d) [a)]	Nominal: 0.0573 (95% confidence limits: 0.050-0.0657) **Mean measured: 0.0567 (95% confidence limits: 0.0491-0.0654)**						
$NOEC_{overall}$ (21 d)	Nominal: 0.0282 **Mean measured: 0.0276**						

CV=coefficient of variation

* Statistically significant effects compared to the pooled control (ANOVA followed by Dunnett's test; $p<0.05$).

[a)] EC_{10} based on cumulative offspring per female data.

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia pulex* the NOEC of BAS 750 F was determined to be 0.0276 mg a.s./L. The EC_{10} value was 0.0567 mg a.s./L, based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint are a NOEC of 0.0276 mg a.s./L and an EC_{10} of 0.0567 mg a.s./L based upon nominal concentrations. The RMS notes tests for significance between the controls was not reported, although there does not appear to be any discrepancies between the solvent control and the water control that would be deemed significant. The method of analysis was confirmed as fully validated (III CA B.5.1.2.6).

The agreed endpoints suitable for use in the risk assessment are:
NOEC of 0.0276 mg a.s./L and an EC_{10} of 0.0567 mg a.s./L (mm)

Report:	B.9.2.5/4 Dinehart S., 2016 a BAS 750 F: Life-cycle toxicity test of the saltwater mysid, *Americamysis bahia*, conducted under flow-through conditions 2016/7001293
Guidelines:	EPA 850.1350
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no.: COD-001740; purity: 98.8%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Americamysis bahia*) juveniles aged less than 24 hours old and sourced from an in-house culture.

Test design: Flow-through system (28 d); 5 test substance concentrations plus a control and a solvent control (10µL dimethylformamide/L). 3 replicates for each test substance concentration, the control and the solvent control each with 30 mysids per glass aquaria (15 mysids per retention chamber) making a total of 90 per test concentration. Mysids were maintained in retention chambers until sexual maturity. At time of sexual maturity (day 13) male-female pairs were transferred into brood cups. Remaining mysids (after isolation of male-female pairs) were pooled and placed in seven separate brood cups within glass aquaria. Dead parental mysids and juveniles released during the test were removed daily. Survival and symptoms of toxicity were assessed daily and assessment of reproduction (number of offspring produced by each female) from day 14 on, determination of length of F0 mysids on day 14 and 28 and F1 mysids after 12d of exposure; determination of F0 dry weight at test termination.

Endpoints: NOEC based on survival, reproductive success (offspring per female and days to first brood release), length and dry weight.

Test concentrations: Control (dilution water), solvent control (10µL dimethylformamide/L); 1.0, 2.0, 4.0, 8.0 and 16µg BAS 750 F/L (nominal), corresponding to mean measured concentrations of <LOQ (control), <LOQ (solvent control), 0.931, 1.50, 3.42, 6.57 and 13.2µg a.s./L.

Preparation: Diluter stock solutions prepared at a target concentration of 1.6g a.s./L by dissolving 0.1619 or 0.3239g of the test substance in 0.10 or 0.2L of DMF respectively. A Hamilton Model 600 syringe dispenser was used to serially dilute the stock solution into the test solutions.

Test conditions: The test chambers were glass aquaria (19.3 L, 19×39 -78×21cm) with 2 cylindrical retention baskets per test chamber, one for reproduction observation and the other for growth observation. Retention baskets consisted of a glass Petri dish base (approximately 1.5 × 15cm) with a stainless steel screen collar (mesh with an approximate mesh opening of 381µm). Brood cups consisting of a glass Petri dish base (approximately 1.5 × 10cm) with the same type of stainless steel screen collar; stainless steel screens were attached to the Petri dish with translucent silicone sealant.

The dilution water was commercial sea salt mix (Crystal Sea Marinemix; Marine Enterprises International, Inc. Baltimore, Maryland) added to laboratory freshwater. The flow rate was approximately 8 volume exchanges/aquarium/24 hours. Mysids were fed with live brine shrimps (*Artemia* sp.) three times daily, with the exception of the first and last days of the study when mysids were fed twice, in combination with a commercial enrichment mixture and rotifers once per day. Aeration was initiated following day 21 as the dissolved oxygen concentrations fell below 60%

Salinity:	19.2-21.0‰;
Temperature:	24.6-26.2°C;
pH:	7.8-8.2;
Oxygen content:	4.0-7.8 mg/L (57-108%);
Photoperiod:	14h light: 10h dark with 30min transition periods;
Light intensity:	479-652lux;

Analytics: Analytical verification of test substance concentrations was conducted using a LC-method with MS/MS detection. The minimum quantifiable limit was 0.250 µg a.s/L.

Statistics: Statistical comparison of the control and solvent control data with test substance treatments using SAS software. Dunnett's test and Williams' test for determination of NOEC values (α=0.05) when assumptions on normality and homogeneity of variance were met, otherwise non-parametric analysis was used.

II. RESULTS AND DISCUSSION

All the validity criteria (EPA 850.1350(1996)) were met:

- ≤25% of first generation control females fail to produce young (0%)
- ≥3 young produced per control female per day (20.1)

Analytical verification of test substance concentrations was conducted in each concentration at test initiation and after 7, 14, 21, and 28 days. Measured concentrations for BAS 750 F were between 80% and 90% of nominal at test initiation. Measured concentrations after 7, 14 and 21 days ranged from 75% to 92%, from 69% to 102% and from 76% to 90% of nominal, respectively. At test termination, measured concentrations were between 75% and 102% of nominal. The following biological results are based on mean measured concentrations.

No statistically significant (and biologically relevant) differences were determined between the control and the solvent control data. Thus, data of the test substance treatments were compared to the dilution water control. There was no statistically significant reduction in survival, reproduction, growth and time of first brood release for any test substance treatment. The results are summarised in Table B.9.2.5/4-1.

Table B.9.2.5/4-1: Chronic toxicity (28 d) of BAS 750 F to saltwater mysids (*Americamysis bahia*)

Concentration [µg a.s./L] (nominal)	**Control**	**Solvent control**	**1.0**	**2.0**	**4.0**	**8.0**	**16**
Concentration [µg a.s./L] (mean measured)	**--**	**--**	**0.931**	**1.50**	**3.42**	**6.57**	**13.2**
Survival on day 28 of the first generation (F0) [%]	93	86	87	93	84	85	93
Survival on day 12 of the second generation (F1) [%]	96	87	93	98	93	98	98
F0: Reproductive success [offspring per female] (± SD)	20.1 ± 4.32	19.1 ± 3.44	27.4 ± 4.00	25.7 ± 1.92	29.7 ± 4.57*	28.7 ± 2.16	30.4 ± 1.57
F0: Days to first brood release (± SD)	16.3 ± 0.378	16.3 ± 0.143	15.4 ± 0.378	15.2 ± 0.360	15.5 ± 0.192	15.3 ± 0.515	15.4 ± 0.143
F0: Mean body length on day 28, males (± SD) [mm]	6.03 ± 0.190	6.12 ± 0.102	6.10 ± 0.0431	6.28 ± 0.0595	6.12 ± 0.107	6.24 ± 0.100	6.35 ± 0.0266
F0: Mean body length on day 28, females (± SD) [mm]	6.37 ± 0.103	6.40 ± 0.0729	6.67 ± 0.0512	6.65 ± 0.0436	6.57 ± 0.0389	6.68 ± 0.0648	6.68 ± 0.0715
F0: Mean dry weight on day 28, males (± SD) [mg]	0.960 ± 0.0254	0.944 ± 0.0664	0.954 ± 0.0110	1.09 ± 0.0223	1.02 ± 0.0826	1.07 ± 0.0783	1.06 ± 0.0710
F0: Mean dry weight on day 28, females (± SD) [mg]	1.36 ± 0.0557	1.37 ± 0.0877	1.64 ± 0.0479	1.53 ± 0.0950	1.53 ± 0.0595	1.60 ± 0.0755	1.55 ± 0.118
F1: Mean body length after 12 days of exposure, males (± SD) [mm]	4.82 ± 0.196	4.98 ± 0.142	4.93 ± 0.149	5.06 ± 0.0381	5.03 ± 0.181	4.92 ± 0.169	4.96 ± 0.119
F1: Mean body length after 12 days of exposure, females (± SD) [mm]	4.87 ± 0.241	5.12 ± 0.192	5.09 ± 0.113	5.34 ± 0.0714	5.16 ± 0.218	5.13 ± 0.222	5.12 ± 0.0818
Endpoints [µg BAS 750 F/L] (mean measured)							
NOEC $_{overall}$ (28 d)	≥ 13.2						

SD=standard deviation

*One female died on day 14, prior to releasing any young and this female was consequently excluded from analysis

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for BAS 750 F was determined to be ≥ 13.2µg a.s./L based on mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is a NOEC of ≥13.2µg a.s./L. The RMS notes that the oxygen concentration should be maintained within 60-105% rather than 57-108%. Given the small deviation and additional attempts were made to account for this in the study and no adverse effects were observed in the control, this deviation is not expected to have negatively affected the test. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
NOEC of ≥13.2µg a.s./L (mm)

B.9.2.6 Toxicity to sediment dwelling invertebrates

Report:	B.9.2.6/1 Clark R., 2015 a BAS 750 F-10-day toxicity test exposing midge (*Chironomus dilutus*) to a test substance applied to sediment under static-renewal conditions 2015/7000621
Guidelines:	EPA 850.1735
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: *Chironomus dilutus* third instar larvae, aged 9-10 days old at test initiation, and sourced from Smithers Viscient culture.

Test design: Semi-static system (10 days) with renewal of overlying water for 5 test concentrations plus a water control and a solvent control (acetone) with 11 replicates per test substance concentration and for the controls (8 replicates for biological response of test organisms and 3 replicates for chemical analysis). 10 larvae were used per test vessel (80 per concentration). Survival, abnormal behaviour and growth (body weight) were assessed daily.

Endpoints: NOEC and LC_{50}/EC_{50} (regarding survival and growth).

Test concentrations: Water control, solvent control, 6.3, 13, 25, 50 and 100.0 mg BAS 750 F/kg dry sediment (nominal), corresponding to mean measured sediment concentrations of 7.2, 13, 22, 53 and 97 mg BAS 750 F/kg dry sediment. All solutions were observed to be clear and colourless with no visible undissolved test substance.

Preparation: A 20 mg/mL stock solutions was prepared by dissolving 1.0120g a.s. to 50 ml of acetone, which was then split into 5 individual dosing stocks. 10 ml of each stock was applied to 50g of fine silica sand, and the solvent allowed to evaporate. The sand was then added to 1.75kg of sediment using a jar-rolling technique.

Test conditions: 300 mL glass beakers with 100 mL (approximately 4cm layer) spiked wet artificial sediment (according to OECD 218), 175 mL laboratory well water; the overlying water was initially renewed by adding two volume additions per test vessel per day using delivery system and water-distribution system (days -1 to 3: 350 mL per day and test vessel, days 4 to 6: 700 mL per day and test vessel, days 7 to 10: 1050 mL per day and test vessel); no aeration; daily feeding of ground flaked fish food suspended in laboratory well water (4.0 mg/mL), 1.5 mL of food suspension per test vessel.

pH:	6.4-7.2;
Oxygen:	3.5-7.3 mg/L;
Total hardness:	68-80 mg $CaCO_3$/L;
Conductivity:	470-530µS/cm;
Ammonia:	≤0.10-0.44 mg/L;
Water temperature:	22-23°C;
Light intensity:	280lux-800lux;
Photoperiod:	16h light: 8h dark;

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with MS/MS detection. The limit of quantitation was 0.0972 mg/kg.

Statistics: Shapiro-Wilk's Test for normality; Wilcoxon's Rank Sum Two-Sample Test and Unequal Variance Two-Sample t-Test (α=0.05) for comparison of the survival and growth data in the control groups; Dunnett's Multiple Comparison for comparison of the growth data and Steel's Many-One Rank Sum Test for comparison of survival in the treatment groups to the negative control (α=0.05). CETIS™ Version 1.8 was the software used to perform statistical computations.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 218 (2004):

- ≤10% control mortality (4%)

Analytical verification of test substance concentrations in the sediment, the overlaying water and the pore water were conducted in each concentration at day 0 and day 10. Recoveries in the sediment were 90-110% of the nominal concentrations at test initiation and 76-94% after 10 days, and in the pore water ranged from 0.016-0.34%. The following biological results are based on the mean measured sediment concentrations.

Following 10 days of exposure, midge survival in both the control and solvent control averaged 96%. During the same period, midge growth in the control and solvent control averaged 2.16 and 2.05 mg ash-free dry weight per larva, respectively. At test termination, a significant difference was determined in survival among midge larvae exposed to the 53 and 97 mg/kg treatment level when compared to the water control. Statistical analysis of the relevant treatment levels determined a significant difference in growth among midge larvae exposed to the 13 and 22 mg/kg sediment dry weight treatment level compared to the control. The results are summarised in Table B.9.2.6/1-1.

Table B.9.2.6/1-1: Effects of BAS 750 F on survival and growth of *Chironomus dilutus* (10 d)

Concentration [mg BAS 750 F/kg dry sediment] (nominal)	**Water control**	**Solvent control**	**6.3**	**13.0**	**25.0**	**50.0**	**100.0**
Concentration [mg BAS 750 F/kg dry sediment] (mean measured)	--	--	**7.2**	**13.0**	**22.0**	**53.0**	**97.0**
Mean survival (10 d) [%] (SD)	96 (7)	96 (5)	96 (5)	94 (7)	95 (8)	79 (10) *	84 (7) *
Mean ash-free dry weight per larva (10 d) [mg] (SD)	2.16 (0.64)	2.05 (0.18)	1.90 (0.48)	1.56 (0.50) #	1.34 (0.37) #	2.05 (0.34) &	1.73 (0.38) &

	Endpoints [mg BAS 750 F/kg dry sediment] (mean measured)
LC_{50}/EC_{50} (growth and survival)	> 97
NOEC (midge survival)	22
NOEC (midge growth , ash-free dry weight)	7.2

SD: standard deviation

* Significant difference compared to the control, based on Steel's Many-One Rank Sum Test (α=0.05).

\# Significant difference compared to the control, based on Dunnett's Multiple Comparison Test (α=0.05).

& Based on the survival effect observed, this treatment level was excluded from statistical analysis of growth (ash free dry weight per larva) for the determination of the NOEC value.

III. CONCLUSION

In a 10-day semi-static sediment test with *Chironomus dilutus* the LC_{50} and EC_{50} values of BAS 750 F for survival and growth were determined to be both ≥ 97 mg/kg dry sediment based on mean measured concentrations.

RMS Comment: The RMS notes that arithmetic mean measured concentrations rather than geometric mean measured concentrations have been used to calculate the endpoints, resulting in the endpoints slightly underestimating toxicity. Consequently the endpoints have been recalculated using geometric mean measured concentration so that the EC_{50} is >96 mg a.s./kg dry sediment, the NOEC for midge survival is 22 mg a.s./kg dry sediment and the NOEC for midge growth is 7.08 mg a.s./kg dry sediment. The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an EC_{50} of >96 a.s./L and a NOEC of 7.08 mg a.s./L. The RMS notes that the test guidelines EPA 850.1735 (1996) is intended for testing saltwater mysids rather than *Chironomus* species. Additionally, dissolved oxygen levels nearly fell below (3.5 mg/L) the acceptable levels according to EPA 850.1735, although increasing the flow rate appears to have mediated this. The minimum light intensity was 280lux rather than the minimum of 500lux recommended in EPA 850.1735 (1996), although given there were no observed adverse effects on the controls, this deviation is not expected to have adversely affected the study. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
NOEC of 7.08 mg a.s./kg dry sediment (mm)

Report:	B.9.2.6/2 Backfisch K.,Weltje L., 2015a Chronic toxicity of Reg.No. 5924326 (M750F003; metabolite of BAS 750 F) to the non-biting midge *Chironomus riparius*-a spiked sediment study 2015/1003916
Guidelines:	OECD 218 (2004)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F003 (metabolite of BAS 750 F), batch no. L84-250, purity: 99.6%, beige powder.

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*) first instar larvae from in-house culture (non-GLP), originally obtained from "the Zoological Institute of the J.W. Goethe University" Frankfurt, Germany.

Test design: Static system (28 days) for 5 test concentrations plus a solvent (acetone) control and a water control with 4 replicates per test substance concentration and for the water control, and 6 replicates for the solvent control. 20 larvae were added to each vessel. Emergence rate and development rate were assessed.

Endpoints: NOEC and EC_{50} (regarding emergence rate and development rate).

Test concentrations: Solvent control, water control, 0.200, 0.400, 0.800, 1.600 and 3.200 mg M750F003/kg dry sediment (nominal), corresponding to initial measured concentrations of 0.125, 0.272, 0.504, 0.983 and 1.944 mg/kg dry sediment. The stock solution was clear.

Preparation: A stock solution was prepared by dissolving 20.88 mg of the test substance in 20.88mL acetone. Test sediment was prepared by mixing relevant aliquots of the stock with 80g of quartz sand, allowing the solvent to evaporate and then carefully added to the sediment.

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), overlaid with 400 mL M4 water (Elendt medium) with gentle aeration and TetraMin food provided at 0.5 mg food/larva/day. The test vessels were allowed to stabilise for 2 days before the addition of the larvae. One additional vessel for each treatment group was set up for chemical analysis on DAT 2 for each treatment group. The DAT 30 analysis was conducted in the same test vessels as used for the biological assessments.

pH:	7.70-8.06;
Oxygen:	7.97-9.44 mg/L;
Total hardness:	2.48mmol/L;
Conductivity:	667µS/cm;
Alkalinity:	0.83mmol/L
Temperature:	20.3-20.7°C;
Light intensity:	550-950lux;

	Photoperiod: 16h light: 8h dark;
Analytics:	Analytical verification of test substance concentrations was conducted using an HPLC-method with MS-detection. For the overlaying water the limit of quantification was 0.001 mg/L and the limit of detection was 0.00025 mg/L. For sediment the limit of detection was 0.015 mg/kg dry sediment.
Statistics:	ANOVA followed by Williams' Multiple sequential t-test for determination of the NOEC based on emergence and development rate (α=0.05); Probit and Weibull analysis using linear max for determination of EC_x values. ToxRatPro version 2.10 was used to perform statistical calculations.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 218 (2004)):

- ≥70% control emergence (91.25%)
- ≥60% oxygen concentration of saturation (minimum 7.70 mg/L)
- Less than ±1.0°C variation in water temperature (0.4°C variation)

Analytical verification of test substance concentrations in the overlying water, the pore water and the sediment was conducted in the controls and in each concentration at the beginning and the end of the test. Recoveries in the sediment were in the range between 60.8% and 68.0% of the nominal concentrations at test initiation and between 20.6% and 28.8% of nominal at test termination. Overlying water concentrations ranged from 0.0119 to 0.212 mg/L at test start and from 0.0209 to 0.363 mg/L at test end. The pore water concentrations ranged from 0.061 to 1.050 mg/L at test start and from 0.0215 to 0.384 mg/L at test termination. The analytical results show that M750F003 has quite a low affinity for sediment and quickly redistributed into the pore water and the overlying water. To quantify the redistribution, mass balance calculations were made on basis of the measured amounts in sediment, overlaying water and pore water. On test initiation approximately 35% of the test substance was found in pore water and overlaying water. The mass balance on DAT 2 shows, that the total recovery in the whole system was between 95.9% and 104.3%. This confirms the correct application of the test substance on test initiation. Therefore, the following biological results are based on the initial measured sediment concentrations.

On DAI 13 (= day after insertion of the larvae), the first emerged midges were observed. Male midges emerge before females, and there was no indication for different effects on male and female test organisms. Therefore the male and female data were pooled for the calculations. No statistically significant differences compared to the control treatments were found for emergence rate and development rate up to and including the highest test substance. Hence, all endpoints (EC_x and NOEC) are equal or above the highest test concentration. The results are summarised in Table B.9.2.6/2-1.

Table B.9.2.6/2-1: Effects of M750F003 (metabolite of BAS 750 F) on emergence and development of *Chironomus riparius*

Concentration [mg M750F003/kg dry sediment] (nominal)	**Control**	**Solvent control**	**0.200**	**0.400**	**0.800**	**1.600**	**3.200**
Concentration [mg M750F003/kg dry sediment] (initial measured)	**--**	**--**	**0.125**	**0.272**	**0.504**	**0.983**	**1.944**
Emergence rate (ER) (28 d) [#]	0.9125 ± 0.0479	0.8750 ± 0.0524	0.9125 ± 0.0854	0.8500 ± 0.1354	0.9000 ± 0.0707	0.9250 ± 0.1190	0.7875 ± 0.0629
Development rate (DR) (28 d) [#]	0.0701 ± 0.0012	0.0689 ± 0.0016	0.0694 ± 0.0003	0.0683 ± 0.0004	0.0686 ± 0.0015	0.0685 ± 0.0015	0.0699 ± 0.0005
Endpoints [mg M750F003/kg dry sediment] (initial measured)							
EC_{50} emergence & development rate (28 d)	> 1.944						
EC_{10} emergence & development rate (28 d)	> 1.944						
NOEC emergence & development rate (28 d)	≥ 1.944						

[#] Values represent mean and standard deviation from all replicates, each with 20 larvae.

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC of M750F003 (metabolite of BAS 750 F) was determined to be ≥ 1.944 mg a.s./kg dry sediment based on emergence and development rate (initial measured).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an EC_{50} >1.944 mg/kg, an EC_{10} >1.944 mg/kg and a NOEC of ≥1.944 mg/kg. The method of analysis was confirmed as fully validated for aquatic medium and for sediment (III CA B.5.1.2.6).

The agreed endpoints suitable for use in the risk assessment are:
NOEC of ≥1.944 mg L (im)

Report: B.9.2.6/3
Backfisch K.,Weltje L., 2015b
Chronic toxicity of Reg.No. 5834378 to the non-biting midge *Chironomus riparius*- a spiked sediment study
2014/1243181
Guidelines: OECD 218 (2004)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%, appearance: white powder

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*) larvae, sourced from in-house culture (non-GLP, originally obtained from "Zoological Institute of the J.W. Goethe University", Frankfurt am Main, Germany).

Test design: Static system (28 days) for 5 test concentrations plus a solvent (acetone) control and a water control of 4 replicates per test substance concentration and for the water control and 6 replicates for the solvent control. Twenty larvae were added to each test vessel. Emergence rate and development rate were assessed.

Endpoints: NOEC, EC_{50} (regarding emergence rate and development rate).

Test concentrations: Solvent (acetone) control, water control, 0.075, 0.150, 0.300, 0.600 and 1.200 mg a.s./kg dry sediment (nominal), corresponding to initial measured concentrations of 0.0755, 0.155, 0.303, 0.557 and 1.158 mg a.s./kg dry sediment. The stock solution was clear.

Preparation: A stock solution was prepared from 22.33 mg of the test substance in 44.12mL of acetone. Aliquots of the stock solution were added to 80g quartz sand and the acetone allowed to evaporate for an hour. The spiked sand was then added to 620g sediment, which was then adjusted to 30% moisture content and 100g of the spiked sediment added to each beaker.

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), overlaid with 400 mL M4 water (Elendt medium) with gentle aeration and TetraMin food provided at 0.5 mg food/larva/day. The test vessels were allowed to stabilise for 2 days before the addition of the larvae. One additional vessel for each treatment group was set up for chemical analysis on DAT 2 for each treatment group. The DAT 30 analysis was conducted in the same test vessels as used for the biological assessments.

pH 7.63-8.26;
Oxygen content: 7.36-8.79 mg/L;
Total hardness: 2.45mmol/L;
Conductivity: 613µS/cm;
Water temperature: 19.7-20.3°C;
Light intensity: 520-980lux;
Photoperiod: 16h light: 8h dark;

Analytics: Analytical verification of test substance concentrations in overlaying water and sediment was conducted using a HPLC-method with MS detection. For the overlaying water the limit of quantification was 0.001 mg/L and the limit of detection was 0.00050 mg/L. For sediment the limit of quantification was 0.050 mg/kg dry sediment and the limit of detection was 0.016 mg/kg dry sediment.

Statistics: Williams' Multiple sequential t-test for determination of the NOEC based on emergence and development rate (α=0.05); Probit and Weibull analysis for determination of EC_x values. ToxRatPro version 2.10 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 218 (2004)):

- ≥70% control emergence (96.25%)
- ≥60% oxygen concentration of saturation (minimum 7.36 mg/L)
- Less than ±1.0°C variation in water temperature (0.6°C variation)

Analytical verification of test substance concentrations in the overlying water, the pore water and the sediment was conducted in each concentration at the beginning and the end of the test. Recoveries in the sediment were in a range between 93% and 103% of the nominal concentrations at test initiation and 83% and 89% of nominal at test termination. BAS 750 F concentrations found in the overlaying water ranged from <LOD to 0.007 mg a.s./L at test initiation and from <LOD to 0.0029 mg a.s./L at test end. Measured pore water concentrations were between <LOD and 0.0126 mg a.s./L at the beginning of the test and between <LOD and 0.00233 mg a.s./L at test termination. The following biological results are based on initial measured sediment concentrations.

On DAI 15 (= day after insertion of the larvae), the first emerged midges were observed. No statistically significant differences compared to the control treatments were found for emergence rate and development rate up to and including the highest test substance concentration. Hence, all endpoints (EC_x and NOEC) are equal or above the highest test concentration. The results are summarised in Table B.9.2.6/3-1.

Table B.9.2..6/3-1: Effects of BAS 750 F on emergence and development of *Chironomus riparius*

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**0.075**	**0.150**	**0.300**	**0.600**	**1.200**
Concentration [mg a.s./kg dry sediment] (initial measured)	**--**	**--**	**0.0755**	**0.155**	**0.303**	**0.557**	**1.158**
Emergence rate (ER) [#]	0.9625 ± 0.0479	0.9250 ± 0.0689	0.9125 ± 0.0479	0.9125 ± 0.0629	0.9375 ± 0.0629	0.8500 ± 0.0408	0.8570 ± 0.1190
Development rate (DR) [#]	0.0585 ± 0.0040	0.0606 ± 0.0022	0.0607 ± 0.0039	0.0608 ± 0.0023	0.0596 ± 0.0010	0.0594 ± 0.0014	0.0588 ± 0.0026
Endpoints [mg BAS 750 F/kg dry sediment] (initial measured)							
EC_{50} emergence rate, development rate (28 d)	> 1.158						
EC_{10} emergence rate, development rate (28 d)	> 1.158						
NOEC emergence rate, development rate (28 d)	≥ 1.158						

[#] Values represent mean and standard deviation from all replicates, each with 20 larvae.

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius*, the NOEC value of BAS 750 F was determined to be ≥ 1.158 mg a.s./kg dry sediment (initial measured) based on emergence rate and development rate.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an EC_{50} >1.158 mg a.s./L and a NOEC of ≥1.158 mg a.s./L. The RMS notes that egg masses should be collected four or five days before test initiation according to OECD 218 (2004) rather than three days prior in this study, although no adverse effects were observed in the control, so this deviation is not expected to have adversely affected the study. The method of analysis was confirmed as fully validated for aquatic medium and for sediment (III CA B.5.1.2.6).

The agreed endpoints suitable for use in the risk assessment are:
NOEC of ≥1.158 mg a.s./ kg dry sediment (im)

Report:	B.9.2.6/4 Clark R., 2015 b BAS 750 F-10-Day toxicity test exposing freshwater Amphipods (*Hyalella azteca*) to a test substance applied to sediment under static-renewal conditions 2015/7000622
Guidelines:	EPA 850.1735
GLP:	Yes

I. MATERIAL AND METHODS

Test substance:	BAS 750 F, batch no. COD001740, purity: 98.8%
Test species:	Amphipods (*Hyalella azteca*) aged 8 days old and sourced from laboratory cultures maintained at Smithers Viscient.
Test design:	Semi-static system (10 days) for 5 test concentrations plus a solvent (acetone) control and a water control of 8 replicates for the biological response of test organisms and 3 replicates for chemical analysis, with 10 amphipods per test vessel. Survival and abnormal behaviour were assessed daily and dry weight was determined at test end.
Endpoints:	NOEC and LC_{50}/EC_{50} (regarding survival and growth).
Test concentrations:	Control (dilution water), solvent control (acetone), 6.3, 13, 25, 50 and 100 mg BAS 750 F/kg dry sediment (nominal), corresponding to mean measured concentrations of 6.6, 12, 22, 50 and 100 mg BAS 750 F/kg dry sediment. The stock solution and dosing solutions were clear and colourless with no visible undissolved test substance.
Preparation:	A stock solution was prepared by dissolving 1.0163g of a.s. to 100 ml with acetone. Dilutions of this stock were prepared to 25ml from aliquots of the stock solution and acetone. 15mL of each stock was applied to 50g of fine silica sand, and the solvent allowed to evaporate. The sand was then added to 1.75kg of sediment using a jar-rolling technique for four hours at 15 rpm then allowed to equilibrate for a 27 day period with weekly mixing to ensure the sediment was homogenous.
Test conditions:	300 mL glass vessels with 100 mL spiked artificial sediment (OECD 218, 1.9% C_{org} and pH 7.5) and 175 mL overlaying laboratory well water. The overlying water was renewed by adding 350 ml per test vessel per day using an intermittent delivery system. 1.5 mL of yeast, cereal leaves and flaked fish food suspension was added for food daily.
	pH: 6.7-7.5;

Oxygen content:	2.6 mg/L-8.4 mg/L;
Total hardness:	68 – 80 mg $CaCO_3$/L;
Total alkalinity:	20 – 24 mg $CaCO_3$/L;
Conductivity: 380 – 480µS/cm;	
Ammonia:	≤ 0.10-0.44 mg/L (as nitrogen);
Water temperature:	22-23°C;
Photoperiod:	16 hours light: 8 hours darkness;
Light intensity:	210 – 520lux;

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with MS/MS detection. The limit of quantitation was 0.56 mg/kg on day 0 and 0.49 mg/kg on day 10 for sediment, 0.011 mg/kg on both days for pore water and 0.0011 mg/kg on both days for overlying water.

Statistics: Shapiro-Wilk's Test for normality; Wilcoxon's Rank Sum Test and Equal Variance Two-Sample t-Test (both α=0.05) for comparison of control and solvent control data; Steel's Many-One Rank Sum Test for determination of NOEC values (α=0.05). Statistical analysis was performed with CETIS™ Version 1.8.

II. RESULTS AND DISCUSSION

All the acceptability criteria (EPA 850.1735) were met:

- ≥80% mean control survival (96% for the control and 98% for the solvent control)
- 40-100% dissolved oxygen saturation (minimum 2.6 mg/L)

Analytical verification of test substance concentration was conducted in each concentration at test initiation and at test termination. Measured concentrations of BAS 750 F in sediment ranged from 99%-120% of nominal test substance concentrations at test initiation and from 76% to 97% of nominal at test termination. Overlaying water concentrations of BAS 750 F were between 0.015 and 0.20 mg a.s./L on day 0 and between 0.012 and 0.17 mg a.s./L on day 10. Measured pore water concentrations of BAS 750 F ranged from 0.12 to 2.0 mg a.s./L on day 0 and from 0.076 to 1.4 mg a.s./L on day 10. The following biological results are based on mean measured concentrations.

After 10-days of exposure, statistical analysis determined no significant difference in survival and growth among amphipods exposed to any of the treatment levels tested compared to the water control. The results are summarised in Table B.9.2.6/4-1.

Table B.9.2.6/4-1: Effect of BAS 750 F on survival and growth of *Hyalella azteca*

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**6.3**	**13**	**25**	**50**	**100**
Concentration [mg a.s./kg dry sediment] (mean measured)	**--**	**--**	**6.6**	**12**	**22**	**50**	**100**
Mean survival (SD) (10 d) [%]	96 (5)	98 (5)	100 (0)	98 (7)	100 (0)	100 (0)	95 (11)
Mean individual dry weight (SD) (10 d) [%]	0.20 (0.024)	0.20 (0.027)	0.20 (0.020)	0.18 (0.013)	0.19 (0.011)	0.18 (0.010)	0.18 (0.045)
Endpoints [mg a.s./kg dry sediment] (mean measured)							
LC_{50}/EC_{50} (10 d)	> 100						
NOEC (10 d)	≥ 100						

SD=Standard Deviation

III. CONCLUSION

In a 10-day semi-static acute sediment test with *Hyalella azteca* the LC_{50}/EC_{50} of BAS 750 F was determined to be >100 mg BAS 750 F/kg dry sediment based on mean measured concentrations. The NOEC was ≥ 100 mg a.s./kg dry sediment (mean measured).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an EC_{50} >100 mg a.s./kg dry sediment and a NOEC of ≥100 mg a.s./ kg dry sediment. The RMS notes that the conditions of the overlying water varied and were not equal to the ranges expected for reconstituted water. In particular, conductivity was 380-480µS/cm although it should range over 330-360µS/cm according to 850.1735. Given that no negative effects were observed in the controls, the overlying water is not expected to have adversely affected the test. Additionally, the length of the test organisms should be measured and considered with growth, although as the NOEC is ≥100 mg a.s./L and there is no indication effects to length in the report, although as there has been no effect on weight, it is not expected that length would have been significantly affected. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoint suitable for use in the risk assessment is:
NOEC of ≥100 mg a.s./ kg dry sediment (mm)

Report: B.9.2.6/5
Clark R., 2015c
BAS 750 F-10-Day toxicity test exposing estuarine amphipods (*Leptocheirus plumulosus*) to a test substance applied to sediment under static conditions
2015/7000623
Guidelines: EPA 850.1740
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Marine Amphipod (*Leptocheirus plumulosus*) juveniles, 2-4mm in length at test initiation, sourced from Chesapeake Cultures, Inc., Hayes, VA, USA.

Test design: Static system (10 days) for 5 test concentrations plus control and solvent control with 9 replicates per test substance concentration and for the controls (5 replicates for biological response of test organisms and 4 replicates for chemical analysis and monitoring of water quality), 20 amphipods per replicate; daily assessment of survival and abnormal behaviour.

Endpoints: NOEC and LC_{50}.

Test concentrations: Control (dilution water), solvent control (acetone), 6.3, 13, 25, 50 and 100 mg BAS 750 F/kg dry sediment (nominal), corresponding to mean measured concentrations of 6.7, 12, 25, 48 and 95 mg BAS 750 F/kg dry sediment. All stock solutions were clear and colourless with no visible undissolved test substance.

Preparation: A 12 mg/L primary stock solution was prepared by adding 0.61535g of BAS 750 F to 50 ml of acetone. Individual stock solutions were prepared by adding aliquots of primary stock to additional acetone 10 ml of the relevant stock was mixed with 50g of fine silica sand for two minutes and the acetone allowed to evaporate over 30 minutes, then added to the sediment with a jar rolling technique for 4 hours at 15 rpm. Following this, the jars were allowed to equilibrate for a 27 day period with weekly mixing to ensure the sediment was homogenous.

Test conditions: 1000 mL glass beakers; 175 mL sediment (natural sediment collected from Sequim Bay, Port Gamble, Washington; 3.2% organic carbon, particle size distribution: 37% sand, 37% silt and 26% clay, pH 7.8) and 725 mL overlying water (filtered natural seawater), total overlying water/sediment volume: approximately 900 mL. There was continuous illumination of light intensity 600-880lux, gentle aeration and no feeding over the test duration.

<u>Overlying water:</u>

Salinity:	20-22‰;
pH	7.2-8.4;
Oxygen content:	5.7 mg/L-7.9 mg/L;
Water temperature:	24-25°C;
Ammonia:	1.1-1.9 mg/L as nitrogen.

<u>Pore water:</u>

Salinity:	24-28‰;
pH	7.0-7.5;
Ammonia:	0.65-12 mg/L as nitrogen.

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with MS/MS detection. The limit of quantitation was 53µg/L for sediment, and 11.4µg/L for pore water and overlying water.

Statistics: Wilcoxon's Rank Sum Two-Sample Test (α=0.05) for comparison of control and solvent control data; Steel's Many-One Rank Sum Test for determination of NOEC value (α=0.05). CETIS version 1.8 was the software

used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the acceptability criteria were met (EPA 850.1740(1996)):

- ≥60% (4.4 mg/L) dissolved oxygen (minimum 5.7 mg/L)
- ≥90% surviving test organism recovery (100%)

Analytical verification of test substance concentration was conducted in each concentration at test initiation and at test termination. Measured concentrations of BAS 750 F in sediment ranged from 93%-110% of nominal test substance concentrations at test initiation and from 93% to 100% of nominal at test termination. Overlaying water concentrations of BAS 750 F were between <0.012 mg a.s./L and 0.13 mg a.s./L at day 0 and between 0.038 mg a.s./L and 0.46 mg a.s./L at day 10. Pore water concentrations of BAS 750 F ranged from 0.058 mg a.s./L to 1.0 mg a.s./L at day 0 and from 0.071 mg a.s./L to 0.65 mg a.s./L at day 10. The biological results are based on the mean measured sediment concentrations.

After 10 days of exposure amphipod survival in both the control and solvent control averaged 100%. No statistically significant differences were detected for amphipod survival up to and including the highest tested concentration compared to the water control. The results are summarised in Table B.9.2.6/1-1.

Table B.9.2.6/1-1: Effect of BAS 750 F on survival of *Leptocheirus plumulosus*

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**6.3**	**13**	**25**	**50**	**100**
Concentration [mg a.s./kg dry sediment] (mean measured)	**--**	**--**	**6.7**	**12**	**25**	**48**	**95**
Mean survival (SD) [%]	100 (0)	100 (0)	98 (1)	99 (1)	99 (1)	100 (0)	98 (1)
	Endpoints [mg BAS 750 F/kg dry sediment] (mean measured)						
LC_{50}	> 95 (95% confidence limits: n.d. [#])						
NOEC	≥ 95						

SD=Standard Deviation

[#] 95% confidence limits could not be calculated as LC_{50} value was empirically determined.

III. CONCLUSION

In a 10-day static acute sediment test with *Leptocheirus plumulosus* the NOEC of BAS 750 F was determined to be ≥ 95 mg a.s./kg dry sediment based on mean measured concentrations. The LC_{50} was >95 mg BAS 750 F/kg dry sediment (mean measured).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an EC_{50} >95 mg a.s./kg dry sediment and a NOEC of ≥95 mg a.s./kg dry sediment. The RMS notes that 800 ml of overlying water (EPA 850.1740(1996)) should be used rather than 725mL, although given all the validity criteria were met and no negative effects were observed in the controls, this deviation is not expected to have adversely affected the study. The method of analysis was confirmed as fully validated for sediment only (III CA B.5.1.2.6).

The agreed endpoints suitable for use in the risk assessment are:
NOEC of ≥95 mg a.s./ kg dry sediment (mm)

B.9.2.7 Effects on algal growth

B.9.2.7.1 Effects on algal growth from the active substance

Report: B.9.2.7.1/1
Brzozowska K., 2014b
BAS 750 F (Reg. No. 5834378) *Pseudokirchneriella subcapitata* SAG 61.81 Growth inhibition test
2013/1250865
Guidelines: OECD 201 (2011), EPA 850.4500
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81. In house culture, originally obtained from the "The Culture Collection of Algae", Göttingen University, Germany.

Test design: Static system (test duration 96 hours) for 5 test concentrations, each with 4 replicates per treatment plus a control with 8 replicates. Growth and changes in morphology were assessed daily. 24h after test duration a recovery experiment was performed over the following 7 days.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours and 96 hours.

Preparation: 20.33 mg of test substance is added to 2033mL AAP medium, ultrasonicated for 10 minutes and shaken for 24h, then passed through a 0.45µm membrane filter.

Test concentrations: Control, 16-fold diluted filtrate, 8-fold diluted filtrate, 4-fold diluted filtrate, 2-fold diluted filtrate and filtrate of the loading of 10 mg BAS 750 F/L, corresponding to geometric mean measured concentrations of <LoD (limit of detection), 0.103, 0.209, 0.416, 0.914, 1.899 mg a.s./L.

Test conditions: Erlenmeyer glass flasks were used as test vessels with test volume 100 mL of AAP medium.

pH:	7.03-8.98;
Temperature:	24.1-24.4°C;
Initial cell density:	1×10^4 cells/mL;
Light intensity:	4363-4480lux;
Shaking:	Mechanical at 90 rpm.

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD detection. The limit of quantitation was 0.01 mg/L and the limit of detection was 0.005 mg/L.

Statistics: Probit analysis for determination of EC_x values; Williams Multiple Sequential t-test Procedure and Multiple Sequentially–rejective U-test after Bonferroni-Holm for determination of the NOEC value (α=0.05). ToxRat Professional 2.10 was the software used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 201(2011)):

- ≥16-fold increase over 72h in biomass (96.8-fold)
- ≤7% control coefficient of variation of the mean specific growth rate at 72h (2.2%)
- ≤35% control mean coefficient of variation for section-by-section growth rate (18.0%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The concentrations of the test substance determined are presented in Table B.9.2.7.1/1-1 below. The test substance concentrations determined in samples collected at exposure termination were in the range of 78.7% to 98.4% of initial concentrations, therefore the following biological results are based on geometric mean measured concentrations.

Table B.9.2.7.1/1-1Concentration and stability of BAS 750 F in the definitive test

Nominal concentration of the test substance (mg/L)	**Mean concentration of the test substance in samples (mg/L)**			**Geometric mean measured concentration* (mg/L)**
	Exposure initiation	Exposure termination	% of initial concentration	
Filtrate of the loading of 10 mg/L	1.914	1.884	98.4	1.899
2-fold dilution filtrate of the loading of 10 mg/L	0.946	0.883	93.3	0.914
4-fold dilution filtrate of the loading of 10 mg/L	0.466	0.372	79.8	0.416
8-fold dilution filtrate of the loading of 10 mg/L	0.235	0.185	78.7	0.209
16-fold dilution filtrate of the loading of 10 mg/L	0.155	0.092	80.0	0.103
Control	<LoD	<LoD	-	-

LoQ = 0.01 mg/L
LoD = 0.005 mg/L
*The geometric mean of the determined test substance concentrations was calculated according to the formula given in the OECD series on testing and assessment No. 23, Annex 2, page 50

No morphological effects on algae were observed in the control and at test substance concentrations of up to and including 0.416 mg a.s./L. At 0.914 mg a.s./L about 15% and 20% of the cells were opalescent after 72 and 96 hours of exposure, respectively, while at 1.899 mg a.s./L about 20% of the cells were opalescent and 20% of the cells were comma-shaped after 72 and 96 hours of exposure. After 72h of exposure, statistically significant effects compared to the control were detected at the four highest and at the five highest test substance concentrations for growth rate and yield, respectively.

After 96h of exposure, statistically significant effects compared to the control occurred at the three highest test substance concentrations for both growth rate and yield. The effects are summarised in Table B.9.2.7.1/1-2.

Table B.9.2.7.1/1-2: Effect of BAS 750 F on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (geometric mean measured)	**Control**	**0.103**	**0.209**	**0.416**	**0.914**	**1.899**
Inhibition in 72 h (growth rate) [%]	0.0	2.0	4.8 *	6.7 *	10.6 *	86.0 *
Inhibition in 72 h (yield) [%]	0.0	9.1 *	20.3 *	26.6 *	39.0 *	99.0 *
Inhibition in 96 h (growth rate) [%]	0.0	0.0	0.6	2.2 *	3.5 *	89.9 *
Inhibition in 96 h (yield) [%] #	0.0	-0.2	2.8	10.9 *	16.6 *	99.6 *
Endpoints [mg BAS 750 F/L] (geometric mean measured)						
E_rC_{50} (72 h)	**1.352 (95% confidence limits: 1.272-1.434)**					
E_rC_{10} (72 h)	0.904 (95% confidence limits: 0.812-0.983)					
E_yC_{50} (72 h)	0.777 (95% confidence limits: 0.606-1.012)					
E_yC_{10} (72 h)	0.215 (95% confidence limits: 0.102-0.315)					
E_rC_{50} (96 h)	1.404 (95% confidence limits: 1.368-1.437)					
E_rC_{10} (96 h)	1.036 (95% confidence limits: 0.990-1.078)					
E_yC_{50} (96 h)	1.163 (95% confidence limits: 1.073-1.268)					
E_yC_{10} (96 h)	0.744 (95% confidence limits: 0.646-0.824)					
NOE_rC (72h)	0.103					
NOE_yC (72h)	<0.103					

Negative values indicate stimulated growth compared to the control.
* Statistically significant differences compared to the control (α=0.05).

The results of the recovery test indicated that algal growth was occurring at the end of the period and that recovery was taking place. Consequently, the BAS 750 F appears to have a reversible effect on growth.

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the respective E_rC_{50} was determined to be 1.352 mg a.s./L (geometric mean measured) after 72 hours of exposure.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an E_rC_{50} 1.352 mg/L, an E_rC_{10} of 0.904 mg/L and a NOE_rC of 0.103 mg a.s./L. The RMS notes that the temperature should be maintained within the range of 21-24°C (OECD 201 (2011)) rather than 24.1-24.4°C. Given that the deviation was only marginally above the limit, all the validity criteria were met and no negative effects were observed in the controls, this deviation is not

expected to have adversely affected the study. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} 1.352 mg/L, an E_rC_{10} of 0.904 mg/L and a NOE_rC of 0.103 mg a.s./L (all mm)

Report:	B.9.2.7.1/2 Bergfield A., 2015a BAS 750 F: Growth inhibition test with the marine diatom, *Skeletonema costatum* 2015/7000620
Guidelines:	EPA 850.4500
GLP:	Yes

Report:	B.9.2.7.1/2a Horn C., 2016a Recalculation of endpoints for the study by Bergfield A., 2015a (BASF DocID 2015/7000620): "BAS 750 F: Growth Inhibition Test with the Freshwater Diatom, *Skeletonema costatum*" 2016/1292092

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system (test duration 96 hours) for 5 test substance concentrations plus a dilution water control and a vehicle control (dimethyl formamide (DMF)) with 4 replicates per concentration and each control group. Growth was assessed daily. Cell density was measured by direct microscopic counting with a haemocytometer.

Endpoints: NOEC, EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.

Test concentrations: Control (dilution water), control (vehicle control (0.050 mL DMF/L), 0.063, 0.13, 0.25, 0.50 and 1.0 mg BAS 750 F/L, corresponding to initial measured concentrations of <LOQ, <LOQ, 0.0529, 0.111, 0.217, 0.434 and 0.876 mg BAS 750 F/L.

Preparation: 0.4049g of a.s. was dissolved in DMF to a volume of 10 ml. This primary standard was serially diluted for the test concentrations

Test conditions: 250 mL Erlenmeyer flasks were filled with a test volume of 50 mL. The test medium was filtered saltwater algal medium, commercial salt mix (Marinemix, Wiegandt GmbH) added to autoclaved ABC reagent water until salinity was 30±2‰. Flasks were swirled by hand once per day.

pH 7.7-8.2;

Temperature:	20.5-20.9°C;
Initial cell densities:	1×10^4 cells/mL;
Photoperiod:	14 hours light: 10 hours dark;
Light intensity:	About 4497-4860lux;

Analytics: Analytical verification of test substance concentrations was conducted using a LC-method with MS/MS detection. Samples were centrifuged for 10 minutes at approximately 3,500rpm before analysis to separate out precipitate. The minimum quantifiable limit was 0.0250 mg/L.

Statistics: A t-test for comparison of control and vehicle control results. Further statistical analyses were conducted using the blank control. ANOVA and one tailed Dunnett's test for determination of the NOEC values (α=0.05). EC_x values were calculated by nonlinear modelling procedure. SAS software (version 9.3) was used to perform statistical analysis.

II. RESULTS AND DISCUSSION

All the validity criteria of EPA 850.4500 (2012) were met:

- ≥30-fold increase over 96h in biomass (16.8-fold)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The concentrations of the test substance determined in samples collected at exposure initiation were in the range of 84% to 88%. The test substance concentrations determined in samples collected at exposure termination were in the range of 67% to 83% of initial concentrations. The biological results were based on geometric mean measured concentrations.

After 72h of exposure, statistically significant effects compared to the control were detected at the three highest test substance concentrations for growth rate and yield. After 96h of exposure, statistically significant effects compared to the control occurred at the two highest test substance concentrations for growth rate and yield. The effects on algal growth are summarised in Table B.9.2.7.1/2-1.

Table B.9.2.7.1/2-1: Effect of BAS 750 F on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Control**	**Vehicle control**	**0.063**	**0.13**	**0.25**	**0.5**	**1**
Concentration [mg a.s./L] (geometric mean measured)	**< LOQ**	**< LOQ**	**0.0490**	**0.0985**	**0.199**	**0.419**	**0.845**
Inhibition in 72 h (growth rate) [%]	--	0	1	0	4 *	15 *	66 *
Inhibition in 72 h (yield) [%] [1)]	--	0	3	-1	11 *	36 *	90 *
Inhibition in 96 h (growth rate) [%] [1)]	--	0	-1	-1	2	19 *	66 *
Inhibition in 96 h (yield) [%] [1)]	--	-1	-5	-3	6	54 *	94 *

Endpoint Type	**Endpoint value (geometric mean measured concentration) mg a.s./L**	**95% confidence interval**
E_rC_{50} (72h)	**0.679**	0.631 – 0.730
E_rC_{20} (72h)	0.458	0.399 – 0.505
E_rC_{10} (72h)	0.373	0.309 – 0.424
E_yC_{50} (72h)	0.479	0.387 – 0.599
E_yC_{20} (72h)	0.318	0.180 – 0.393
E_yC_{10} (72h)	0.257	0.113 – 0.335
E_rC_{50} (96h)	0.676	0.669 – 0.682
E_rC_{20} (96h)	0.423	0.417 – 0.430
E_rC_{10} (96h)	0.331	0.324 – 0.338
E_yC_{50} (96h)	0.399	0.397 – 0.401
E_yC_{20} (96h)	0.268	0.266 – 0.370
E_yC_{10} (96h)	0.218	0.215 – 0.220
NOE_rC (72h)	0.0985	-
NOE_yC (72h)	0.0985	-
NOE_rC (96h)	0.199	-
NOE_yC (96h)	0.199	-

* Significant reduction in growth rate/ yield as compared to the control (Dunnett's test, α=0.05).
[1)] Inhibition compared to control; negative values indicate stimulated growth.

III. CONCLUSION

In a 96 hour static toxicity test with *Skeletonema costatum* the E_rC_{50} of BAS 750 F was determined to be 0.679 mg a.s./L (72 h) based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for use in risk assessments and the endpoints are E_rC_{50} of 0.679 mg/L, an E_rC_{10} of 0.373 mg/L and a NOE_rC of 0.0985 mg/L (all 72h). The RMS notes that changes to the morphology of the cells should be observed and some conditions of the test medium such as hardness and conductivity were not reported. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of 0.679 mg/L, an E_rC_{10} of 0.373 mg/L and a NOE_rC of 0.0985 mg/L (all mm)

Report:	B.9.2.7.1/3 Bergfield A., 2015b BAS 750 F: Growth inhibition test with the freshwater diatom, *Navicula pelliculosa* 2015/7000618
Guidelines:	EPA 850.4500
GLP:	Yes

Report:	B.9.2.7.1/3a Horn C., 2016b Recalculation of endpoints for the study by Bergfield A., 2015b (BASF DocID 2015/7000618): "BAS 750 F: Growth Inhibition Test with the Freshwater Diatom, *Navicula pelliculosa*" 2016/1292093

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: Freshwater diatom, *Navicula pelliculosa*. In house stock originally obtained from the Department of Botany, Culture Collection of Algae, University of Texas at Austin, USA.

Test design: Static system (test duration 96 hours) for 6 test concentrations, each with 4 replicates per treatment plus a control and vehicle control with 6 replicates. Growth was assessed daily.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours and 96 hours.

Test concentrations: Control, vehicle control (0.050 mL DMF/L), 0.22, 0.44, 0.88, 1.8, 3.5 and 7.0 mg BAS 750 F/L, corresponding to initial measured concentrations of <LOQ, <LOQ, 0.176, 0.358, 0.724, 1.47, 2.86 and 3.20 mg a.s./L. All test solutions appeared clear and colourless except at 3.20 mg a.s/L where there were slight amounts of undissolved test substance.

Preparation: A primary standard was created by adding 1.4170g of a.s. with DMF to a volume of 10 ml, then serially diluted with DMF to create a series of 10 ml standard solutions.

Test conditions: The test vessels were 250 mL Erlenmeyer flasks with 100 mL of algal nutrient medium plus silicate.

pH:	7.5-8.4;
Temperature:	22.4-24.1°C;
Initial cell density:	1×10^4 cells/mL;
Light intensity:	3736-4661lux;
Shaking:	100 rpm

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with MS/MS detection. Samples were centrifuged for around 10 minutes at 3,500rmp before analysis to separate out precipitate. The minimum quantifiable limit was 0.0250 mg a.s./L.

Statistics: A Least Significant Difference test by a t-test for comparison of control and vehicle control results. Analyses to establish EC_x and NOEC were conducted using the blank control and nominal test substance treatments ≤ 3.5 mg a.s./L, because undissolved test material was observed in the highest test concentration. One-tailed Dunnett's test for determination of the NOEC values (α=0.05); nonlinear modelling procedure for calculation of EC_x values. SAS version 9.3 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria of EPA 850.4500 (2012) were met:

- ≥100-fold increase in biomass over 96h (155-fold)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The test substance concentrations determined in samples collected at exposure initiation were in the range of 80% and 82% and at test termination in the range of 49% and 64%, except for nominal test concentration 7.0 mg a.s./L, which was 46% and 19% of nominal concentration at test initiation and test termination, respectively. Due to poor initial recovery and undissolved test material in the solution, the nominal 7.0 mg a.s./L treatment level was not used for statistical analysis. The following biological results are based on geometric mean measured concentrations.

Table B.9.2.7.1/3-1: Measured concentrations of BAS 750 F in test solutions

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L) [% of nominal concentration)		
	0-hours[a]	96-hours[b]	Geometric mean
Control	<LOQ[c]	<LOQ[c]	<LOQ[c]
Solvent control	<LOQ[c]	<LOQ[c]	<LOQ[c]
0.22	0.176 [80]	0.107 [49]	0.137 [62]
0.44	0.358 [81]	0.256 [58]	0.303 [69]
0.88	0.724 [82]	0.496 [56]	0.599 [68]
1.8	1.47 [82]	1.15 [64]	1.30 [72]
3.5	2.86 [82]	1.83 [52]	2.29 [65]
7.0	3.20 [46]	1.32 [19]	2.06 [29]

[a] Samples from parent solution.
[b] Samples from composites of replicates A-F for control and A-D for test concentrations
[c] LOQ=0.0250 mg a.s./L

After 72h and 96h of exposure, statistically significant effects compared to the control were detected at test substance concentrations ≥ 0.599 mg a.s./L for both growth rate and yield. The effects on algal growth are summarised in Table B.9.2.7.1/3-2.

Table B.9.2.7.1/3-2: Effect of BAS 750 F on the growth of the diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Control**	**Vehicle control**	**0.22**	**0.44**	**0.88**	**1.8**	**3.5**	**7.0**
Concentration [mg a.s./L] (geometric mean measured)	**< LOQ**	**< LOQ**	**0.137**	**0.303**	**0.599**	**1.30**	**2.29**	**2.06**
Inhibition in 72 h (growth rate) [%]	--	0	0	-1	15*	52*	72*	76*
Inhibition in 72 h (yield) [%] [1)]	--	0	0	0	47*	78*	91*	91*
Inhibition in 96 h (growth rate) [%] [1)]	--	0	0	0	15*	31*	78*	81*
Inhibition in 96 h (yield) [%] [1)]	--	0	0	0	50*	81*	96*	97*

Endpoint Type	**Endpoint value (geometric mean measured concentration) mg a.s./L**	**95% confidence interval**
E_rC_{50} (72h)	**1.347**	**1.162 – 1.565**
E_rC_{20} (72h)	0.682	0.498 – 0.830
E_rC_{10} (72h)	0.478	0.307 – 0.620
E_yC_{50} (72h)	0.671	0.559 – 0.826
E_yC_{20} (72h)	0.438	0.285 – 0.531
E_yC_{10} (72h)	0.351	0.189 – 0.448
E_rC_{50} (96h)	1.577	1.065 – 2.313
E_rC_{20} (96h)	0.952	0.197 – 1.287
E_rC_{10} (96h)	0.732	0.072 – 1.077
E_yC_{50} (96h)	0.654	0.397 – 1.146
E_yC_{20} (96h)	0.392	0.068 – 0.559
E_yC_{10} (96h)	0.300	0.022 – 0.459
NOE_rC (72h)	0.303	-
NOE_yC (72h)	0.303	-
NOE_rC (96h)	0.303	-
NOE_yC (96h)	0.303	-

LOQ=0.0250 mg a.s./L

[#] Negative values indicate stimulated growth compared to the control.

* Statistically significant differences compared to the control (Dunnett's test, p=0.05).

[$] The nominal 7.0 mg a.s./L treatment level was not used for statistical analysis due to poor initial recovery (46%) and undissolved test material in the solution.

III. CONCLUSION

In a 96-hour algae test with *Navicula pelliculosa,* the E_rC_{50} (72 h) for BAS 750 F was determined to be 1.347 mg a.s./L (geometric mean measured).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an E_rC_{50} of 1.347 mg/L, an E_rC_{10} of 0.478 mg/L and a NOE_rC of 0.303 mg/L, all 72h. The RMS notes that changes to the morphology of the cells should be observed and some conditions of the test medium such as hardness and conductivity were not reported. Due to poor initial recovery and undissolved test material in the solution, the nominal 7.0 mg a.s./L treatment level was not used for statistical analysis. However, basing the EC_x values off the other five test concentrations is acceptable. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of 1.347 mg/L, an E_rC_{10} of 0.478 mg/L and a NOE_rC of 0.303 mg/L (all mm)

Report:	B.9.2.7.1/4 Bergfield A., 2015c BAS 750 F: Growth inhibition test with the Cyanobacterium, *Anabaena flos-aquae* 2015/7000617
Guidelines:	EPA 850.4500
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%, appearance: whitish solid.

B. STUDY DESIGN

Test species: *Anabaena flos-aquae*, sourced from in-house cultures, parent stock obtained from University of Texas at Austin (UTEX), USA.

Test design: The study was a static system with test duration 96 hours. 5 test substance concentrations were plus a control and a vehicle control (dimethyl formamide (DMF)) were tested, each with 4 replicates per treatment and control group. Growth was assessed daily.

Endpoints: NOEC, EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.

Preparation: A primary standard was created from 1.0121g of a.s. with DMF to a volume of 10 ml, then serially diluted to 10 ml to create working standards. Aliquots of the working standards were then diluted again to prepare the test solutions.

Test concentrations: Control, vehicle control (0.050 mL DMF/L), 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L (nominal), corresponding to initial measured concentrations of <LOQ, <LOQ, 0.247, 0.507, 1.09, 2.04 and 3.20 mg a.s./L. The test solutions appeared clear and colourless throughout the study.

Test conditions: 250 mL Erlenmeyer flasks with 100 mL of freshwater algal nutrient medium created by adding reagent salts to autoclaved ABC reagent water. Flasks were swirled daily by hand.
pH: 7.6-9.0;
Temperature: 22.2-25.4°C;
Initial cell density: 1×10^4 cells/mL;
Light intensity: 2247-2307lux;

Analytics: Analytical verification of test substance concentrations was conducted using a LC-method with MS/MS detection. Samples were centrifuged for 10 minutes at 3,500rpm before analysis to separate out precipitate. The minimum quantification limit was 0.00250 mg a.s./L.

Statistics: A t-test for comparison of control groups. Statistical analyses were conducted using the blank control. A one-tailed Dunnett´s test was used for determination of the NOEC value ($p<0.05$). EC_{50} values were calculated using logistic (sigmoid-shaped) model fit. SAS software version 9.3 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria of EPA 850.4500 (2012) were met:

- ≥100-fold increase in biomass over 96h (1,140-fold)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The measured values of BAS 750 F ranged from 64% to 84% of nominal at test initiation and from 59% to 71% of nominal at test termination. Geometric mean measured concentrations were 0.233, 0.476, 0.989, 1.85, and 3.08 mg a.s./L.

Table B.9.2.7.1/4-1:Measured concentrations of BAS 750 F in test solutions

Nominal concentration (mg a.s./L)	**Measured concentration (mg a.s./L) [% of nominal concentration)**		
	0-hours	96-hours	Geometric mean
Control	<LOQ[a]	<LOQ[a]	<LOQ[a]
Solvent control	<LOQ[a]	<LOQ[a]	<LOQ[a]
0.31	0.247 [80]	0.220 [71]	0.233 [75]
0.63	0.507 [80]	0.447 [71]	0.476 [76]
1.3	1.09 [84]	0.898 [69]	0.989 [76]
2.5	2.04 [82]	1.67 [67]	1.85 [74]
5.0	3.20 [64]	2.97 [59]	3.08 [62]

[a] LOQ=0.0250 mg a.s./L

The following biological results are based on initial measured concentrations. After 96 hours of exposure, yield was statistically significantly reduced at the highest test substance concentration compared to the control (Dunnett's test, p<0.05). The effects on algal growth are summarised in Table B.9.2.7.1/4-2.

Table B.9.2.7.1/4-2: Effect of BAS 750 F on the growth of *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.31**	**0.63**	**1.3**	**2.5**	**5.0**
Concentration [mg a.s./L] (geometric mean measured)	**< LOQ**	**< LOQ**	**0.233**	**0.476**	**0.989**	**1.85**	**3.08**
Inhibition in 72h (growth rate) [%] #	--	--	-1	0	1	1	0
Inhibition in 72 h (yield) [%] #	--	--	-2	0	4	3	0
Inhibition in 96h (growth rate) [%] #	--	--	-2	-2	-2	1	1*
Inhibition in 96 h (yield) [%] #	--	--	-7	-8	-7	2	5*
	Endpoints [mg a.s./L] (geometric mean measured)						
E_rC_{50}/E_yC_{50} (96h & 72 h)	> 3.08						
E_rC_{10}/E_yC_{10} (96h & 72 h)							
NOE_rC (72 h)	≥3.08						
NOE_rC (96 h)	2.04						
NOE_yC (72 h)	≥3.08						
NOE_yC (96 h)	2.04						

Negative values indicate stimulated growth compared to the control.
* Statistically significant differences compared to the control (Dunnett's test, $p<0.05$).

III. CONCLUSION

In a 96-hour algae test with *Anabaena flos-aquae*, the E_rC_{50} (72 h) value for BAS 750 F was determined to be >3.08 mg a.s./L based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for use in risk assessments and the endpoints are an E_rC_{50} of >3.08 mg/L, an E_rC_{10} of >3.08 mg/L and a NOE_rC of ≥3.08 mg/L, all 72h. The RMS notes that changes to the morphology of the cells should be observed and some conditions of the test medium such as hardness and conductivity were not reported. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of >3.08 mg/L, an E_rC_{10} of >3.08 mg/L and a NOE_rC of ≥3.08 mg/L (all mm)

B.9.2.7.2 Effects on algae from metabolites

Report: B.9.2.7.2/1
Backfisch K., 2015a
Effect of Reg.No. 6003432 (M750F007, metabolite of BAS 750 F) on the growth of the green alga *Pseudokirchneriella subcapitata*
2015/1003914
Guidelines: OECD 201, 850.4500
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F007 (metabolite of BAS 750 F), batch no. L87-32-1, purity: 97.0%, appearance: beige solid

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81. In house culture with fresh strains obtained at least once a year from the "Sammlung von Algenkulturen", Göttingen University, Germany.

Test design: Static system (test duration 72 hours) for 5 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates. Growth was assessed daily and changes in morphology were observed at the end of the test.

Endpoints: EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Preparation: A stock solution was prepared by adding 8.85 mg of the test substance to 885mL of nutrient solution and the pH adjusted to 8.11. Aliquots of the stock solution and algal nutrient solution were taken to prepare the test concentrations.

Test concentrations: Control, 0.625, 1.25, 2.5, 5 and 10 mg M750F007/L. A separate reference test was performed in the laboratory in March 2015 with potassium dichromate and the EC_{50} was 0.539 mg/L.

Test conditions: Test vessels were 100 mL Erlenmeyer dimple flasks with test volume 60 mL and OECD medium;

pH:	7.92-8.04;
Temperature:	22 ± 1°C;
Initial cell density:	1×10^4 cells/mL;
Light intensity:	8000lux;
Shaking:	Constant at 130 rpm

Analytics: Analytical verification of test substance concentrations was conducted using an HPLC-method with MS detection. The limit of quantification was 0.001 mg/L and the limit of detection was 0.00025 mg/L.

Statistics: Probit analysis for determination of EC_x values (α=0.05). ToxRat Professional 2.10 was used to conduct the statistical analysis.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 201(2011)):

- ≥16-fold increase over 72h in biomass (76-fold)
- ≤7% control coefficient of variation of the mean specific growth rate at 72h (3.1%)
- ≤35% control mean coefficient of variation for section-by-section growth rate (22.0%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The concentrations of the test substance determined in samples collected at exposure initiation were in the range of 93% and 99% of nominal concentrations. The test substance concentrations determined in samples collected at exposure termination were in the range of 90% to 94% of nominal concentrations, therefore the following biological results are based on nominal concentrations.

No morphological effects on algae were observed in the control and at all test substance concentrations. After 72h of exposure, no statistically significant effects compared to the control were detected at all test substance concentrations for growth rate and yield. The results are summarised in Table B.9.2.7.2/1-1.

Table B.9.2.7.2/1-1: Effect of M750F007 (metabolite of BAS 750 F) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**0.625**	**1.25**	**2.5**	**5**	**10**
Inhibition in 72 h (growth rate) [%] [#]	--	- 0.5	1.0	1.6	2.4	- 1.7
Inhibition in 72 h (yield) [%] [#]	--	- 1.9	2.9	7.2	6.5	- 7.2
Endpoints [mg M750F007/L] (nominal)						
E_rC_{50} (72 h)	> 10					
E_yC_{50} (72 h)	> 10					
NOE_rC	≥10					
NOE_yC	≥10					

[#] Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} for M750F007 was determined to be >10 mg/L (nominal).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an E_rC_{50} >10 mg/L and a NOE_rC of ≥10 mg/L. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} >10 mg/L and a NOE_rC of ≥10 mg/L (all mm)

Report: B.9.2.7.2/2
Brzozowska-Wojoczek K., 2015a
Reg.No. 6010286 (Metabolite of BAS 750 F, M750F008) *Pseudokirchneriella subcapitata* SAG 61.81 Growth inhibition test
2015/1001491
Guidelines: OECD 201 (2006), EPA 850.4500
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F008 (metabolite of BAS 750 F), batch no. L85-94, purity: 96.5 ± 1%.

B. STUDY DESIGN

Test species: Unicellular green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81. In house culture originally obtained from the "The Culture Collection of Algae", Göttingen University, Germany.

Test design: Static system over a test duration of 96 hours. 6 test concentrations, each with 4 replicates per treatment plus a control and a solvent control with 8 replicates. Growth and changes in morphology were assessed daily.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours and 96 hours.

Preparation: 99.2 mg of the test substance was added to 992µL of DMF solvent. 250µL of the stock was added to AAP medium to a volume of 2500 ml. This solution was sonicated for 15 minutes then filtered through a 0.45µm nitrocellulose membrane filter.

Test concentrations: Control, solvent control (0.1 mL DMF/L). . A filtrate of the loading of 10 mg M750F008/L was used as a test concentration along with five dilutions of the filtrate: 7.6-fold, 5.06-fold, 3.38-fold, 2.25-fold, 1.5-fold. These corresponded to geometric mean measured concentrations of 7.08, 0.83, 1.24, 1.93, 3.08, 4.67 mg/L. The stock solution and all filtered solutions were transparent without undissolved particles.

Test conditions: 250 mL Erlenmeyer glass flasks with test volume 100 mL test medium. AAP medium was used as the test medium.

pH:	7.02-7.36 (initiation) and 7.61-9.06 (termination);
Temperature:	22.9-23.5°C;
Initial cell densities	1×10^4 cells/mL;
Light intensity:	3975-4200lux (constant);
Shaking:	90rmp (constant)

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD detection. The limit of quantification was 0.005 mg/L and the limit of detection was 0.01 mg/L.

Statistics: Student-t-test for comparison of control and solvent control. Shapiro-Wilk's test conformed normal distribution and Levene's test confirmed heterogeneous variances. Probit analysis was performed for determination of EC_x values. The NOEC was determined with the Jonckheere-Terpstra Test or Welch-t-test with Bonferroni-Holm adjustment (α=0.05). ToxRat Professional was the software used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 201(2011)):

- ≥16-fold increase over 72h in biomass (113.2-fold)
- ≤7% control coefficient of variation of the mean specific growth rate at 72h (1.6%)
- ≤35% control mean coefficient of variation for section-by-section growth rate (23.8%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. The concentrations of the test substance determined in samples is presented in Table B.9.2.7/3-1. The test substance concentrations determined in samples collected at exposure termination were in the range of 81.2% to 100.3% of initial concentrations. The following biological results are based on geometric mean measured concentrations.

Table B.9.2.7/3-1-1: Concentration and stability of the test substance

Nominal concentration of the test substance (mg/L)	Mean concentration of the test substance in samples (mg/L)			Geometric mean measured concentration* (mg/L)
	Exposure initiation	Exposure termination	% of initial concentration	
Filtrate of the loading of 10 mg/L	7.07	7.09	100.3	7.08
1.5-fold dilution filtrate of the loading of 10 mg/L	4.74	4.61	97.3	4.67
2.25-fold dilution filtrate of the loading of 10 mg/L	3.16	3.00	94.9	3.08
3.38-fold dilution filtrate of the loading of 10 mg/L	2.09	1.78	85.2	1.93
5.06-fold dilution filtrate of the loading of 10 mg/L	1.38	1.12	81.2	1.24
7.6-fold dilution filtrate of the loading of 10 mg/L	0.91	0.76	83.5	0.83
Solvent control	<LoD	<LoD	-	-
Control	<LoD	<LoD	-	-

LoQ = 0.01 mg/L
LoD = 0.005 mg/L
*The geometric mean of the determined test substance concentrations was calculated according to the formula given in the OECD series on testing and assessment No. 23, Annex 2, page 50

At exposure termination in the test substance concentration of 1.93 mg/L and 3.08 mg/L opalescent rod shaped cells were observed. In the test substance concentrations of 4.67 and 7.08 mg/L cells were bigger, opalescent and rod shaped. In the remaining test substance concentrations no differences in shape, size and colour of algae cells were reported as compared to the algae cells in the control and the solvent control. After 72 and 96h of exposure, statistically significant effects compared to the control were detected at the five highest test substance concentrations for growth rate and yield. The effects on algal growth are summarised in Table B.9.2.7.2/2-2.

Table B.9.2.7.2/2-2: Effect of M750F008 on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg M750F008/L] (geometric mean measured)	**Control**	**Solvent control**	**0.83**	**1.24**	**1.93**	**3.08**	**4.67**	**7.08**
Inhibition in 72 h (growth rate) [%] [b)]	--	0.0	0.8	3.5 *	12.1 *	45.7 *	54.7 *	70.7 *
Inhibition in 72 h (yield) [%] [b)]	--	0.0	4.0	15.6 *	43.5 *	89.0 *	93.0 *	97.2 *
Inhibition in 96 h (growth rate) [%] [a)]	--	0.0	0.1	4.2 *	7.2 *	26.8 *	45.7 *	75.3 *
Inhibition in 96 h (yield) [%] [b)]	--	0.0	0.3	20.5 *	32.6 *	77.3 *	92.2 *	98.7 *

	Endpoints [mg M750F008/L] (geometric mean measured)
E_rC_{50} (72 h)	**4.08 (95% confidence limits: 3.83-4.34)**
E_rC_{10} (72 h)	1.40 (95% confidence limits: 1.33-1.47)
E_yC_{50} (72 h)	2.01 (95% confidence limits: 1.72-2.33)
E_rC_{50} (96 h)	4.78 (95% confidence limits: 4.23-5.38)
E_rC_{10} (96 h)	2.12 (95% confidence limits: 1.92-2.34)
E_yC_{50} (96 h)	2.21 (95% confidence limits: 1.86-2.63)
NOE_rC	0.83
NOE_yC	0.83

* Statistically significant differences compared to the control (α=0.05).
a) Jonckheere-Terpstra Test
b) Welch-t-test with Bonferroni-Holm adjustment

III. CONCLUSION

In a 96-hour static algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} was determined to be 4.08 mg/L and the E_rC_{10} was determined to be 1.40 mg/L (both geometric mean measured) after 72 hours of exposure.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint are an E_rC_{50} of 4.08 mg/L, an E_rC_{10} of 1.40 mg/L and a NOE_rC of 0.83 mg/L. The RMS notes that the light intensity should range from 4440-8880lux rather than 3975-4200lux. Additionally the pH should increase by no more than 1.5 over the course of the test 7.36-8.92 and 7.24-9.06 in the water and solvent control respectively, and in also in multiple test concentrations. As all the validity criteria were met and no negative effects were observed in the controls, these deviations are not expected to have adversely affected the experiment. Additionally Tables B.9.2.7/3-1-1 and B.9.2.7/3-1-2 of the report appears to have mismatched the geometric mean measured concentrations, although the RMS has accounted for this and the error has no effect on the results. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of 4.08 mg/L, an E_rC_{10} of 1.40 mg/L and a NOE_rC of 0.83 mg/L (all mm)

Report: B.9.2.7.2/3
Rzodeczko H., 2016a
Reg.No. 5863469 (metabolite of BAS 750 F, M750F006) *Pseudokirchneriella subcapitata* SAG 61.81 Growth inhibition test
2015/1184815
Guidelines: OECD 201 (2006)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F006 (metabolite of BAS 750 F, Reg. no.: 5863469), batch no. L87-30, purity: 98.9 ± 1%.

B. STUDY DESIGN

Test species: Unicellular green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81. In house culture originally obtained from the "The Culture Collection of Algae", Göttingen University, Germany.

Test design: Static system for a test duration of 72 hours. 6 test concentrations, each with 3 replicates per treatment plus a control and a solvent control with 6 replicates, each. Growth was assessed daily and changes in morphology were observed at test termination.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Preparation: 80.2 mg of the test substance was added to 800µL DMF to make a stock solution. 200µL of the stock was added to AAP medium to 2L. The solution was then sonicated for 15 minutes and filtered through a 0.45µm membrane filter.

Test concentrations: Control, solvent control (0.1 mL DMF/L). A filtrate of the loading of 10 mg M750F006/L was used as a test concentration and then serially diluted by 2.5-fold, 6.25-fold, 15.6-fold, 39.0-fold, 97.7-fold for the remaining test concentrations. These corresponded to geometric mean measured concentrations of 3.159, 1.268, 0.485, 0.200, 0.075 and 7.754 mg a.s./L. All filtrates appeared visually homogenous and transparent without any undissolved particles. A test with the reference substance 3,5-dichlorophenol was performed in the laboratory in June 2015 which had a 72h E_rC_{50} of 2.41 mg/L

Test conditions: 250 mL Erlenmeyer glass flasks were used as test vessels with 100 mL of AAP medium;

pH:	7.20-7.24 at initiation and 7.17-8.82 at termination;
Temperature:	22.4-23.0°C;
Initial cell density:	1×10^4 cells/mL;
Light intensity:	6330-7080lux (continuous);
Shaking:	90rpm (continuous)

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD detection. The limit of quantification was 0.005 mg/L and the limit of detection was 0.001 mg/L.

Statistics: Student-t-test for comparison of control and solvent control. EC_x values were determined by probit analysis. Shapiro-Wilk's test and Levene's test were used to determine normal distribution and homogenous variation respectively. Williams Multiple Sequential t-test Procedure (α=0.05) was used for determination of the NOEC value. ToxRat Professional was the software used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 201(2011)):

- ≥16-fold increase over 72h in biomass (183.0-fold)
- ≤7% control coefficient of variation of the mean specific growth rate at 72h (1.3%)
- ≤35% control mean coefficient of variation for section-by-section growth rate (18.2%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test, and the results of which are presented in Table B.9.2.7.2/3-1 below.

Table B.9.2.7.2/3-1 Concentration and stability of M750F006

Nominal concentration of the test substance (mg/L)	**Mean concentration of the test substance in samples (mg/L)**			**Geometric mean measured concentration* (mg/L)**
	Exposure initiation	Exposure termination	% of initial concentration	
Filtrate of the loading of 10 mg/L	7.676	7.835	102.07	7.754
2.5-fold dilution filtrate of the loading of 10 mg/L	3.144	3.174	100.95	3.159
6.25-fold dilution filtrate of the loading of 10 mg/L	1.264	1.273	100.71	1.268
15.6-fold dilution filtrate of the loading of 10 mg/L	0.477	0.493	103.35	0.485
39.0-fold dilution filtrate of the loading of 10 mg/L	0.201	0.198	98.51	0.200
97.7-fold dilution filtrate of the loading of 10 mg/L	0.086	0.066	76.74	0.075
Solvent control	<LoD	<LoD	-	-
Control	<LoD	<LoD	-	-

LoQ = 0.01 mg/L
LoD = 0.005 mg/L

*The geometric mean of the determined test substance concentrations was calculated according to the formula given in the OECD series on testing and assessment No. 23, Annex 2, page 50

The following biological results are based on geometric mean measured concentrations. At exposure termination all filtrate solutions except the 97.7-fold dilution had deformed cells, and in all 100% of algae were affected except for the 39.0-fold dilution where 80% of the cells were deformed. No morphological effects were observed in the control groups. At the end of the test, statistically significant effects compared to the solvent control occurred at all test substance concentration for both growth rate and yield. The effects on algal growth are summarised in Table B.9.2.7.2/3-2.

Table B.9.2.7.2/3-2: Effect of M750F006 on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg M750F006/L] (geometric mean measured)	**Control**	**Solvent control**	**0.075**	**0.200**	**0.485**	**1.268**	**3.159**	**7.754**
Inhibition in 72 h (growth rate) [%]	0.91	--	2.10 *	17.91 *	48.98 *	57.05 *	58.58 *	63.97 *
Inhibition in 72 h (yield) [%]	4.91	--	10.51 *	61.35 *	92.86 *	95.50 *	95.90 *	97.03 *
Endpoints [mg M750F006/L] (geometric mean measured)								
E_rC_{50} (72 h)	1.424 (95% confidence limits: 0.937 – 2.293)							
E_rC_{10} (72 h)	0.041 (95% confidence limits: 0.009 – 0.095)							
E_yC_{50} (72 h)	0.168 (95% confidence limits: 0.156 – 0.180)							
NOE_rC	<0.075							
NOE_yC	<0.075							

* Statistically significant differences compared to solvent control (Williams Multiple Sequential t-test Procedure, α=0.05).

III. CONCLUSION

In a 72-hour static algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} for M750F006 was determined to be 1.424 mg/L and the E_rC_{10} was determined to be 0.041 mg/L respectively, based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoint is an E_rC_{50} of 1.424 mg/L, an E_rC_{10} of 0.041 mg/L and a NOE_rC of <0.075 mg/L. The RMS notes that the pH should increase by no more than 1.5 over the course of the test, and the pH increased from 7.24-8.82 in the solvent control. As this only occurred in the solvent control, all the validity criteria were met and no negative effects were observed in the controls, this deviation is not expected to have adversely affected the experiment. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of 1.424 mg/L, an E_rC_{10} of 0.041 mg/L and a NOE_rC of <0.075 mg/L (all mm)

Report: B.9.2.7.2/4
Rzodeczko H., 2016 b
Reg.No. 6003433 (metabolite of BAS 750 F, M750F005) *Pseudokirchneriella subcapitata* SAG 61.81 Growth inhibition test
2015/1184816
Guidelines: OECD 201 (2006)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F005 (metabolite of BAS 750 F, Reg. no.: 6003433), batch no. L87-34, purity: 99.4 ± 1%.

B. STUDY DESIGN

Test species: Unicellular green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81. In house culture originally obtained from the "The Culture Collection of Algae", Göttingen University, Germany.

Test design: Static system (test duration 72 hours) for 5 test concentrations, each with 3 replicates per treatment plus a control and a solvent control with 6 replicates each. Growth was assessed daily and changes in morphology were observed at exposure termination.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, solvent control (0.1 mL DMF/L), diluted filtrates of 2-fold, 4-fold, 8-fold, 16-fold and filtrate of the loading of 10 mg M750F005/L, corresponding to geometric mean measured concentrations of 4.273, 2.121, 1.027, 0. 497 and 8.572 mg a.s./L. A test with the reference substance 3,5-dichlorophenol was performed in the laboratory in June 2015 which had a 72h E_rC_{50} of 2.41 mg/L.

Preparation: 85.1 mg test substance added to 850µL of DMF. 200µL of this solution was added to AAP medium up to a total volume of 2L. This stock solution was then sonicated for 15 minutes and filtered through a 0.45µm nitrocellulose membrane filter. The filtrate was visually homogenous and transparent without any undissolved particles.

Test conditions: Test vessels were 250 mL Erlenmeyer glass flasks with 100 mL of AAP medium;

pH:	7.06-7.23 at initiation and 7.67-8.74 at termination;
Temperature:	22.4-23.0°C;
Initial cell density:	1×10^4 cells/mL;
Light intensity:	6460-7160lux (constant);
Shaking:	90rpm (continuously)

Analytics: Analytical verification of test substance concentrations was conducted using an LC-method with DAD detection. The limit of quantification was 0.005 mg/L and the limit of detection was 0.001 mg/L.

Statistics: Student-t-test for comparison of control and solvent control. EC_x values were determined by probit analysis. Shapiro-Wilk's test and Levene's test were used to determine normal distribution and homogenous variation respectively. Williams Multiple Sequential t-test Procedure (α=0.05) was used for determination of the NOEC value. ToxRat Professional was the software used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 201(2011)):

- ≥16-fold increase over 72h in biomass (183.0-fold)
- ≤7% control coefficient of variation of the mean specific growth rate at 72h (1.3%)
- ≤35% control mean coefficient of variation for section-by-section growth rate (18.2%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test, and the results of which are presented in Table B.9.2.7.2/4-1 below.

Table B.9.2.7.2/4-1 Concentration and stability of M750F005

Nominal concentration of the test substance (mg/L)	**Mean concentration of the test substance in samples (mg/L)**			**Geometric mean measured concentration* (mg/L)**
	Exposure initiation	Exposure termination	% of initial concentration	
Filtrate of the loading of 10 mg/L	8.538	8.606	100.80	8.572
2-fold dilution filtrate of the loading of 10 mg/L	4.322	4.225	97.76	4.273
4-fold dilution filtrate of the loading of 10 mg/L	2.190	2.054	93.79	2.121
8-fold dilution filtrate of the loading of 10 mg/L	1.080	0.977	90.46	1.027
16-fold dilution filtrate of the loading of 10 mg/L	0.540	0.458	84.81	0.497
Solvent control	<LoD	<LoD	-	-
Control	<LoD	<LoD	-	-

LoQ = 0.01 mg/L
LoD = 0.005 mg/L
*The geometric mean of the determined test substance concentrations was calculated according to the formula given in the OECD series on testing and assessment No. 23, Annex 2, page 50

The following biological results are based on geometric mean measured concentrations. No morphological effects on algae were observed in the control and at all test substance concentrations. No statistically significant effects were observed between the control groups. At the end of the test, statistically significant effects compared to the solvent control occurred at the highest test substance

concentration for both growth rate and yield. The effects on algal growth are summarised in Table B.9.2.7.2/4-2.

Table B.9.2.7.2/4-2: Effect of M750F005 on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg M750F005/L] (geometric mean measured)	**Control**	**Solvent control**	**0.497**	**1.027**	**2.121**	**4.273**	**8.572**
Inhibition in 72 h (growth rate) [%] [1)]	0.91	--	0.72	- 0.85	2.40	0.58	3.76 *
Inhibition in 72 h (yield) [%] [1)]	4.91	--	3.77	- 4.96	11.71	3.14	18.10 *

	Endpoints [mg M750F005/L] (geometric mean measured)
E_rC_{50} (72 h)	> 8.572
E_rC_{10} (72 h)	> 8.572
E_yC_{50} (72 h)	> 8.572
NOE_rC	4.273
NOE_yC	4.273

* Statistically significant differences compared to solvent control (Williams Multiple Sequential t-test Procedure, α=0.05).
1) Inhibition compared to solvent control; negative values indicate stimulated growth.

III. CONCLUSION

In a 72-hour static algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} for M750F005 was determined to be >8.572 mg/L and the NOE_rC of 4.273 mg/L, based on geometric mean measured concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an E_rC_{50} >8.572 mg/L and an NOE_rC of 4.273 mg/L The RMS notes that the pH should increase by no more than 1.5 over the course of the test, and the pH increased from 7.24-8.82 in the solvent control, and from 7.06-8.62 in the 4-fold dilution. As all the validity criteria were met and no negative effects were observed in the controls, this deviation is not expected to have adversely affected the experiment. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of >8.572 mg/L, an E_rC_{10} of >8.572 mg/L and a NOE_rC of 4.273 mg/L (all mm)

Report: B.9.2.7.2/5
Backfisch K., 2016
Effect of Reg. No. 5924326 (M750F003, metabolite of Bas 750 F) on the growth of the green alga *Pseudokirchneriella subcapitata*
2016/1289875
Guidelines: OECD 201 (2006)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: M750F003 (metabolite of BAS 750 F, Reg. no.: 5924326), batch no. L84-250, purity: 99.6%.

B. STUDY DESIGN

Test species: Unicellular green alga, *Pseudokirchneriella subcapitata*, (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81. In house culture originally obtained from the "The Culture Collection of Algae", Göttingen University, Germany.

Test design: Static system (test duration 72 hours) for 5 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates.

Endpoints: EC_{50} and EC_{10} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control and nominal concentrations 1.23, 3.7, 11.1, 33.3 and 100 mg M750F003/L. A test with the reference substance potassium dichromate was performed in the laboratory in September 2016 and had a 72h E_rC_{50} of 0.799 mg/L.

Preparation: A stock solution was prepared by adding 66.09 mg of M750F003 to 660.9mL nutrient solution. The solution was filter-sterilised using a filter-system, smallest pore size 0.2µm. Solutions were corrected to pH 8.1 with NaOH.

Test conditions: Test vessels were 100 mL Erlenmeyer dimple flasks with 60 mL of nutrient solution according to OECD 201;

pH:	7.66-7.79 at termination;
Temperature:	22±1°C;
Initial cell density:	1×10^4 cells/mL;
Light intensity:	8000lux (continuous);
Shaking:	130rpm (constant)

Analytics: Analytical verification of test substance concentrations was conducted using HPLC/MS. The limit of quantification was 0.001 mg/L and the limit of detection was 0.0002 mg/L.

Statistics: EC_x values were determined by probit analysis. ToxRat Professional 2.10 was the software used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria were met (OECD 201(2011)):

- ≥16-fold increase over 72h in biomass (77-fold)
- ≤7% control coefficient of variation of the mean specific growth rate at 72h (4.3%)
- ≤35% control mean coefficient of variation for section-by-section growth rate (34.5%)

Analytical verification of test substance concentrations was conducted in each concentration at the beginning and at the end of the test. Recover ranged from 97-109% at test initiation and 102-106% at test termination.

The following biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and at all test substance concentrations. The effects on algal growth are summarised in Table B.9.2.7.2/5-1.

Table B.9.2.7.2/5-1: Effect of M750F003 on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg M750F003/L] (nominal)	**Control**	**1.23**	**3.7**	**11.1**	**33.3**	**100**
Inhibition in 72 h (growth rate) [%] [1)]	--	0.8	0.4	3.6	3.0	14.8
Inhibition in 72 h (yield) [%] [1)]	--	4.4	3.1	11.5	11.8	47.1
			Endpoints [mg M750F003/L] (nominal)			
E_rC_{50} (72 h)	> 100					
E_rC_{10} (72 h)	64.11					
E_yC_{50} (72 h)	> 100					
E_yC_{10} (72 h)	14.04					

* Statistically significant differences compared to solvent control (Williams Multiple Sequential t-test Procedure, α=0.05).
1) Inhibition compared to control

III. CONCLUSION

In a 72-hour static algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} for M750F003 was determined to be >100 mg/L and the EC_{10} of 64.11 mg/L, based on nominal concentrations.

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes and the proposed endpoints are an E_rC_{50} >100 mg/L and an EC_{10} of 64.11 mg/L. The RMS notes that the NOEC was not statistically determined, so the chronic risk must be based on the EC_{10} value alone. Additionally temperature, light intensity and initial pH were not measured; for these environmental conditions, the reported values are uncertain. As all the validity criteria were met and no negative effects were observed in the controls, this is not expected to have adversely affected the experiment. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of >100 mg/L, an E_rC_{10} of 64.11 mg/L (all mm)

B.9.2.8. Effects on aquatic macrophytes

Report: B.9.2.8/1
Swierkot A., 2014a
BAS 750 F (Reg. No. 5834378) *Lemna gibba* CPCC 310 growth inhibition test
2014/1001322
Guidelines: OECD 221 (2006), EPA 850.4400 (2012)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8% ± 1.0%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba*), specification CPCC 310, inoculum from 7 days old cultures, sourced from cultures maintained in-house. The stock was originally obtained from "Canadian Phycological Culture Centre (CPCC)", Department of Biology, University of Waterloo, Canada.

Test design: Static system (7 days) for 6 treatment groups (5 test substance concentrations plus control) with 4 replicates for the test substance treatments and 8 replicates for the control. Each replicate had 4 colonies with 3 fronds each added, making the total number of fronds at test initiation 12 per replicate. Growth and morphological effects were assessed on days 3, 5 and 7.

Endpoints: NOEC, EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.

Test concentrations: Control and a 16-fold, 8-fold, 4-fold and 2-fold diluted filtrate and filtrate of the loading of 10 mg BAS 750 F/L, corresponding to time-weighted mean measured concentrations of 0.119, 0.233, 0.485, 0.921 and 1.894 mg a.s./L. The filtered stock solution and the test concentrations during exposure appeared homogenous and transparent. A reference test with 3,5-dichlorophenol was performed in a separate test in the laboratory in December 2013 and resulted in an 7 day E_rC_{50} of 10.4 mg/L

Preparation: 20X AAP medium was added to 41.03 mg a.s up to a volume of 4100 ml. As there were undissolved particles, solutions underwent 10 minutes of ultrasonication and 60rmp mechanical shaking for 24 hours, followed by filtration through a 0.45µm nitrocellulose membrane. Test solutions were then prepared by sequential dilution.

Test conditions: 600 ml glass beakers and lids with 400 mL of test medium,

pH:	7.58-7.79 (initiation) 9.40-9.45 (termination);
Temperature:	24.1-24.6°C,
Light intensity:	6510-6700lux, mean 6626.9lux

Analytics: Analytical verification of the test substance was conducted using an LC-method with DAD detection. The limit of quantification was 0.01 mg/L and the limit of detection was 0.005 mg/L.

Statistics: Probit analysis was used for determination of the EC_x values, and Williams Multiple Sequential t-test Procedure (α=0.05) for determination of the NOEC values. Shapiro-Wilk's Test and Levene's Test were performed to test for normal distribution and homogeneity of variances respectively. ToxRat professional 2.10 was used to perform statistical analyses.

II. RESULTS AND DISCUSSION

All the validity criteria (OECD 221 (2006)) were met:

- <2.5day control frond doubling time (2.03days)

Analytical verification of test substance concentrations was conducted in each test concentration at the beginning and at the end of the test. The concentrations of the test substance determined in samples collected at exposure initiation were: Control (< LoD), 0.132, 0.259, 0.541, 0.998 and 2.017 mg a.s./L. The test substance concentrations determined in samples collected at exposure termination were in the range of 80.04% to 88.10% of initial concentrations. This confirms that the test substance concentrations were stable under test conditions and that the concentration of the test substance remained within ±20% of initial concentrations. The following biological results are based on the initial measured concentrations of the test substance.

The duckweed population in the control vessels showed exponential growth, increasing from 12 fronds per vessel to an average of 131.9 fronds per vessel. The dry weight increased to an average of 22.4 mg per vessel in the control at test termination. No morphological effects were observed up to and including the highest test substance concentration during test duration. No statistically significant differences compared to the control were observed at any test substance concentration for all measured parameters. Effects on growth rate and yield are summarised in Table B.9.2.7/1-1.

Table B.9.2.7/1-1: Effect of BAS 750 F on the growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (initial measured)	**Control**	**0.132**	**0.259**	**0.541**	**0.998**	**2.017**
Inhibition after 7d [%] [#] (growth rate based on frond no.)	0.0	0.1	-1.7	-4.5	-1.0	0.3
Inhibition after 7d [%] [#] (growth rate based on dry weight)	0.0	0.0	-2.8	-8.5	-1.3	-0.9
Inhibition after 7d [%] [#] (yield based on frond no.)	0.0	0.3	-4.5	-12.4	-2.6	0.7
Inhibition after 7d [%] [#] (yield based on dry weight)	0.0	0.0	-7.5	-23.0	-3.5	-2.0
Endpoints [mg BAS 750 F/L] (initial measured)						
E_rC_{50} (7 d) based on frond no.	**> 2.017**					
E_rC_{10} (7 d) based on frond no	> 2.017					
E_yC_{50} (7 d) based on frond no	> 2.017					
E_yC_{10} (7 d) based on frond no	> 2.017					
E_rC_{50} (7 d) based on dry weight	**> 2.017**					
E_rC_{10} (7 d) based on dry weight	> 2.017					
E_yC_{50} (7 d) based on dry weight	> 2.017					
E_yC_{10} (7 d) based on dry weight	> 2.017					
NOEC overall	≥ 2.017					

[#] Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba,* the E_rC_{50} for BAS 750 F was determined to be >2.017 mg a.s./L based on both frond number and dry weight (initial measured concentration).

RMS Comment: The study is considered acceptable and suitable for risk assessment purposes, and the proposed endpoints are an E_rC_{50} of >2.017 mg a.s./L, an E_rC_{10} of >2.017 mg/L and a NOEC of ≥2.017 mg a.s./L. As the final measured concentrations are within ±20% of the initial concentrations (minimum 80.04%), it is acceptable to base the results off the initial measured concentrations. Consequently initial measured concentrations have been used for endpoint values rather than the time weighted average endpoints proposed in the report. The RMS notes that the pH increased by more than the recommended 1.5 over the course of the study (7.70-9.53 in the control, although the pH increased by >1.5 in all test concentrations. Given all the validity criteria were met and no negative effects were observed in the controls, this deviation is not expected to have adversely affected the experiment. The method of analysis was confirmed as validated (III CP B.5.1.2).

The agreed endpoints suitable for use in the risk assessment are:
E_rC_{50} of >2.017 mg/L, an E_rC_{10} of >2.017 mg/L and a NOE_rC of ≥2.017 mg/L (all im)

B.9.2.6. Further testing on aquatic organisms

No additional data on aquatic organisms was submitted, and no literature data was considered relevant to be included in this section

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

Summaries of the studies submitted in support of this application with regard to toxicity to bees are included below and further summarised in Table B.9.3.1-1.

Table B.9.3.1-1: Summary of endpoints of BAS 750 F and BAS 750 01 F to honeybees and bumblebees

Substance	Endpoint	Value	Reference (BASF DocID)
Studies on adult honeybees			
BAS 750 F	48 h acute oral LD_{50}	> 100 µg a.s./bee	B.9.3.1/1 Franke M., 2015a
	48 h acute contact LD_{50}	> 100.0 µg a.s./bee	
	10 d chronic LD_{50}	> 110.5 µg a.s./bee/day	B.9.3.1/5 Kleebaum K., 2015a
	10 d chronic LC_{50}	> 2.562 g a.s./kg food	
	10 d chronic NOED	≥ 110.5 µg a.s./bee/day	
	10 d chronic NOEC	≥ 2.562 g a.s./kg food	
BAS 750 01 F	48 h oral LD_{50}	409.6 µg/bee	B.9.5.1/2 Franke M., 2015a
	96 h contact LD_{50}	296.4 µg/bee	
Studies on honeybee larvae			
BAS 750 F	8 d LD_{50}	43.9 µg a.s./larva	B.9.3.1/6 Kleebaum K., 2015b
	8 d NOED	29.7 µg a.s./larva	
	8 d LC_{50}	1.295 g a.s./kg food	
	8 d NOEC	0.875 g a.s./kg food	
	21 d ED_{50}	> 50.1 µg a.s./larva	B.9.3.1/7 Royer S., 2015 a*
	21 d EC_{50}	> 325 mg a.s./kg food	
	21 d NOED	≥ 50.1 µg a.s./larva	
	21 d NOEC	≥ 325 mg a.s./kg food	
Studies on adult bumblebees			
BAS 750 F	96 h oral LD_{50}	> 195.4 µg a.s./bee	B.9.3.1/2 Amsel K., 2015a **
	96 h contact LD_{50}	> 200.0 µg a.s./bee	

***Study not considered reliable enough for use in the risk assessment.**
****Studies provided as additional information but not used in risk assessment.**

Report: B.9.3.1/1
Franke M., 2015a
Acute toxicity of BAS 750 F to the honeybee *Apis mellifera* L. under laboratory conditions
2015/1128674
Guidelines: OECD 213 (1998)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species:	*Apis mellifera* L. Buckfast (honeybee), young adult worker bees (about 3-5 weeks old) derived from a healthy and queen-right colony, source: BioChem agrar GmbH, Gerichshain, Germany; collected from the top of the bee hive in the morning prior to use.
Test design:	In a 48 hour test, young adult worker bees of *Apis mellifera* L. were exposed orally to BAS 750 F via food (50% (w/v) aqueous sucrose solution with 1 % v/v acetone and 1 % v/v Tween®80). In total, 3 treatment groups were set up (5 dose rates of the test substance, 2 untreated control groups (50 % w/v sucrose solution alone, 50 % w/v sucrose solution with 1 % v/v acetone and 1 % v/v Tween®80) and 4 dose rates of the reference item) with 3 replicates per treatment and 10 bees per replicate. The bees were starved for approximately 1 hour prior to the introduction of the test substance. Assessment of bee mortality and behavioural effects were conducted after 4, 24 and 48 hours.
Endpoints:	Mortality (LD_{50}), behavioural impairments.
Reference item:	Dimethoate EC 400 (dimethoate, 400 g/L nominal).
Test doses:	BAS 750 F: 6.2, 12.5, 25.0, 50.0 and 100.0 µg a.s./bee, resulting in an actual uptake of 6.2, 12.5, 25.0, 50.0 and 100.0 µg a.s./bee. These were prepared by serial dilution of a stock solution of the highest test concentration (100 µg a.s./bee). To measure the consumed amount of control, reference item and test substance solution, the initial feeding tubes were weighed before giving to the bees and then after 1.5 hours (when they were visibly empty). Reference item: 0.069, 0.106, 0.163 and 0.250 µg dimethoate/bee.
Test conditions:	Temperature: 24.0°C – 26.5°C; relative humidity: 45%-67%; photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution. Disposable cardboard cages were used (95 x 50 x 65 mm), with a glass plate at the front and holes in the bottom for ventilation. The bees were fed *ad libitum* with 50 % w/v sucrose solution once the treated feed was fully consumed.
Statistics:	Multiple sequentially-rejective Fisher Test after Bonferroni-Holm for mortality data (one-sided greater, $\alpha = 0.05$); Probit maximum likelihood regression for calculation of the LD_{50} value. Statistical program used was ToxRat Professional 3.1 (2015).

II. RESULTS AND DISCUSSION

After 48 hours, no mortality occurred in the control groups fed with either pure sucrose solution or sucrose solution containing 1 % acetone and 1 % tween.
In the test substance treatment, no mortality occurred after oral consumption of 6.2, 12.5, 25.0, 50.0 and 100.0 µg a.s./bee, after 48 hours. No test substance induced behavioural effects were observed. The results are summarized in Table B.9.3.1/1-1.

Table B.9.3.1/1-1: Toxicity of BAS 750 F to *Apis mellifera* L. (honeybee) in an oral toxicity test

Treatment	Dosage [consumed]	Mortality [%]		
		4 h	24 h	48 h
Control	Sucrose solution	0.0	0.0	0.0
	Acetone-Tween sucrose solution	0.0	0.0	0.0
BAS 750 F [µg a.s./bee]	6.2	0.0	0.0	0.0
	12.5	0.0	0.0	0.0
	25.0	0.0	0.0	0.0
	50.0	0.0	0.0	0.0
	100.0	0.0	0.0	0.0
Reference Item [µg a.s./bee]	0.250	0.0	83.3*	96.7*
	0.163	0.0	53.3*	60.0*
	0.106	0.0	23.3*	30.0*
	0.069	0.0	0.0	0.0
Endpoint [µg consumed a.s./bee]				
LD_{50} (48 h)	> 100.0			

*Significant difference in pairwise comparison between treatment and Tween control (Multiple sequentially-rejective Fisher Test after Bonferroni-Holm for mortality data; $\alpha = 0.05$; one sided greater).

The LD_{50} value (24 h) for the reference item was determined to be 0.171 µg dimethoate/bee (95% confidence limits: 0.154-0.189 µg dimethoate/bee), based on consumption.

Validity Criteria:
The study meets all the validity criteria specified in OECD 213:

- Average control mortality was less than 10 % (being 0 % in both controls)
- The LD_{50} of the toxic standard meets the specified range of 0.1-0.35 µg a.s./bee (being 0.171 µg a.s./bee)

III. CONCLUSION

In an acute oral toxicity study with BAS 750 F on honeybees, the LD_{50} value (48 h) was determined to be > 100 µg a.s./bee.

RMS Comments
The study was carried out according to GLP and follows the guideline OECD 213 with no significant deviations. It was noted that the reported relative humidity (45-67 %) fell below guideline recommendations (50 – 70 %), though as the study validity criteria were met this is not considered to have had a significant effect on the outcome of the study. It was also noted that the control treatments

included a 50 % w/v sucrose solution only and a 50 % w/v sucrose solution with 1 % v/v Tween®80 and 1 % v/v acetone. For completeness it would have been appropriate to include controls consisting of the sucrose solution with separate solvents. However, as there was zero mortality in the two controls and the treated groups, this is not considered to be important by the RMS evaluator.

The agreed endpoint considered suitable for use in the risk assessment is:

48 h LD_{50} (oral) = >100 µg a.s./bee

Report:	B.9.3.1/2 Amsel K., 2015a Acute toxicity of BAS 750 F to the bumblebee *Bombus terrestris* L. under laboratory conditions 2014/1275250
Guidelines:	OECD 213 (1998), OECD 214 (1998), Hanewald *et al.* (2013), Van der Steen (1996), Van der Steen (2001)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F; batch no.: COD-001740; analysed purity: 98.8%.

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected on the morning prior to use. The bees used in the test ranged between 150 and 300 mg in weight. The bees were acclimatized to test conditions for 1 hour and starved for an additional 2 hours prior to the introduction of the test substance.

Test design: In a 96-hour test, adults of *Bombus terrestris* were exposed to 5 doses of BAS 750 F in treated food (50% (w/v) sucrose solution including 1% acetone). In total, 4 treatment groups were set up: 5 dose rates of the test substance, 2 control groups and 4 dose rates of the reference item with 30 replicates per dose and 1 bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were conducted after 4, 24, 48, 72 and 96 hours.

Endpoints: Mortality, behavioural impairments.

Reference item: BAS 152 11 I (dimethoate, nominal 400.0 g/L).

Test doses: Sucrose control (50% (w/v) sucrose solution), sucrose control (50% (w/v) sucrose solution including 1% acetone); reference item at dose rates of 0.25, 0.45, 0.82 and 1.50 µg dimethoate/bumblebee; test substance at dose rates of 12.5, 25.0, 50.0, 100.0 and 200.0 µg BAS 750 F/bumblebee (resulting in an actual uptake of 12.2, 24.7, 48.3, 97.7 and 195.4 µg BAS 750 F/bumblebee). The series of test concentrations were prepared by serial dilution of a stock solution of the highest test concentration. Each bee received 40 µL of the test solution. The actual consumed dose was calculated by weighing the food tubes before and after the consumption period (approximately 4 hours).

Test conditions: Temperature: 24.8 °C – 25.2 °C, relative humidity: 59.2% – 61.0%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution provided *ad libitum*. The bees were kept individually in Nicot cages (7 x 2 cm height x diameter)

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$). Probit analysis using linear weight regression for calculation of the LD_{50} values for the reference item. Statistical calculations were performed using the computer program ToxRat Professional 3.0 beta (2014).

II. RESULTS AND DISCUSSION

After 96 hours of oral exposure, no mortality occurred in the controls or any treatment group. No behavioural effects of surviving bumblebees occurred in all tested dose rates in the oral toxicity test when compared to the control. The results are summarised in Table B.9.3.1/2-1.

Table B.9.3.1/2-1: Toxicity of BAS 750 F to *Bombus terrestris* (bumblebee) in an oral toxicity test

Treatment	Dosage	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	Sucrose	0.0	0.0	0.0	0.0
	Sucrose + 1% acetone	0.0	0.0	0.0	0.0
BAS 750 F [µg a.s./bumblebee]	12.2	0.0	0.0	0.0	0.0
	24.7	0.0	0.0	0.0	0.0
	48.3	0.0	0.0	0.0	0.0
	97.7	0.0	0.0	0.0	0.0
	195.4	0.0	0.0	0.0	0.0
Reference Item [µg dimethoate/bumblebee]	1.45	93.3*	93.3*	100*	100*
	0.78	63.3*	63.3*	63.3*	66.7*
	0.43	50.0*	50.0*	50.0*	50.0*
	0.24	3.3	3.3	3.3	3.3
Endpoint [µg a.s./bumblebee]					
LD_{50} (96 h)	> 195.4				
Reference item LD_{50} (96 h)	0.53 (48 h LD_{50} = 0.56)				

*Significant difference in pairwise comparison between treatment and acetone control (Fisher's Exact Binominal test with Bonferroni correction for mortality data ($\alpha = 0.05$, one sided greater).

III. CONCLUSION

In an acute oral toxicity study with BAS 750 F on bumblebees, the LD_{50} value (96 h) was estimated to be > 195.4 µg BAS 750 F/bumblebee.

RMS Comments
The study was carried out according to GLP. Currently no official guideline for acute toxicity of an active substance/formulation to bumblebees is in circulation, therefore the HSE evaluator has made reference to the (yet to be noted) EFSA guidance, as well as the supplemental papers referred to by the applicant, in assessing the study. With reference to available guidance, no significant deviations were noted. No guidance was available regarding the appropriate sensitivity range for the reference item Dimethoate, although research by Hanewald *et al.* (2014) indicates oral 96 h LD_{50} of approximately 0.2-2.4 µg/bee (mean 1.2 µg/bee). The study report states an appropriate range for the oral test as between 0.25 and 1.50 µg a.s./bee (not referenced, lending a degree of uncertainty to this range) and the 48 h and 96 h LD_{50} both fall within this range (being 0.56 and 0.53 µg a.s./bee, respectively). Altogether this suggests that this batch of bees could be quite sensitive, but this sensitivity falls within available ranges. No mortality occurred in the controls, which meets the validity criteria in OECD 213 and EFSA guidance (<10 % average control mortality). On balance the RMS considers the study to be reliable.

The agreed endpoint is:

96 h LD_{50} (oral) = >195.4 µg a.s./bumblebee

Report:	B.9.3.1/3 Franke M., 2015b Acute toxicity of BAS 750 F to the honeybee *Apis mellifera* L. under laboratory conditions 2015/1128674
Guidelines:	OECD 214 (1998)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8%.

B. STUDY DESIGN

Test species: *Apis mellifera* L. Buckfast (honeybee), young adult worker bees (about 3 – 5 weeks old) derived from a healthy and queen-right colony, source: BioChem agrar GmbH, Gerichshain, Germany; collected from the top of the bee hive in the morning of use.

Test design: In a 48 hour test, young adult worker bees of *Apis mellifera* L. were exposed to 5 dose rates of BAS 750 F in an appropriate carrier (pure acetone) placed on the dorsal bee thorax. In total, 3 treatment groups were set up (5 dose rates of the test substance, 3 untreated control groups and 4 dose rates of the reference item) with 3 replicates per treatment and 10 bees per replicate. Assessment of bee mortality and behavioural effects were done after 4, 24 and 48 hours.

Endpoints:	Mortality (LD_{50}), behavioural impairments.
Reference item:	Dimethoate EC 400 (dimethoate, 400 g/L nominal).
Test doses:	BAS 750 F: 6.2, 12.5, 25.0, 50.0 and 100.0 µg a.s./bee. A stock solution was prepared at the highest test concentration (0.506 g BAS 750 F in 10 mL acetone); from this stock solution the lower test substance concentrations were prepared by step-wise serial dilution in acetone. Control groups: water control (deionised water), Tween control (deionized water + 1.0% v/v wetting agent (Tween®80)) and acetone control. Reference item: 0.106, 0.141, 0.188 and 0.250 µg dimethoate/bee (diluted in 1 % v/v Tween®80). Before application the bees were briefly anaesthetised (20 s) with CO_2. The treatments were applied in 2 µL droplets using an Eppendorf Micropipette.
Test conditions:	Temperature: 24.0°C – 26.5°C; relative humidity: 45%-67%; photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution provided *ad libitum* after topical application of the test substance. Disposable cardboard cages were used (95 x 50 x 65 mm), with a glass plate at the front and holes in the bottom for ventilation.
Statistics:	Descriptive statistics; Multiple sequentially-rejective Fisher Test after Bonferroni-Holm for mortality data (one-sided greater, $\alpha = 0.05$); Probit maximum likelihood regression for calculation of the LD_{50} value of the reference item.

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, no mortality occurred in the control groups treated with either deionized water, tween solution or acetone.
In the test substance treatment, no mortality occurred after thoracic application of 6.2, 12.5, 25.0, 50.0 and 100.0 µg a.s./bee, after 48 hours. No test substance induced behavioural effects were observed. The results are summarized in Table B.9.3.1/3-1.

Table B.9.3.1/3-1: Toxicity of BAS 750 F to *Apis mellifera* L. (honeybee) in a contact toxicity test

Treatment	Dosage [applied]	Mortality [%]		
		4 h	24 h	48 h
Control	Water	0.0	0.0	0.0
	Tween	0.0	0.0	0.0
	Acetone	0.0	0.0	0.0
BAS 750 F [µg a.s./bee]	6.2	0.0	0.0	0.0
	12.5	0.0	0.0	0.0
	25.0	0.0	0.0	0.0
	50.0	0.0	0.0	0.0
	100.0	0.0	0.0	0.0
Reference Item (Dimethoate EC 400) [µg a.s./bee]	0.250	0.0	80.0*	86.7*
	0.188	0.0	43.3*	56.7*
	0.141	0.0	16.7	20.0
	0.106	0.0	0.0	0.0
Endpoint [µg a.s./bee]				
LD_{50} (48 h)	> 100.0			

*Significant difference in pairwise comparison between treatment and tween control (Multiple sequentially-rejective Fisher test after Bonferroni-Holm for mortality data (α = 0.05, one sided greater).

The LD_{50} value (24 h) for the reference item was determined to be 0.195 µg dimethoate/bee (95% confidence limits: 0.180- 0.212 µg dimethoate/bee) in the contact toxicity test.

Validity Criteria
The study meets the validity criteria specified in OECD 214:

- Average control mortality was less than 10 % in all cases (being 0 % in all cases)
- The LD_{50}-24 h of the toxic standard (0.195 µg a.s./bee) meets the specified range (0.1-0.3 µg a.s./bee)

III. CONCLUSION

In an acute contact toxicity study with BAS 750 F on honeybees the LD_{50} value (48 h) was determined to be > 100.0 µg a.s./bee.

RMS Comments
The study was carried out according to GLP and follows the guideline OECD 214 with no significant deviations. It was noted that the reported relative humidity (45-67 %) fell below guideline recommendations (50 – 70 %), though as the study validity criteria were met this is not considered to have had a significant effect on the outcome of the study. Although the study uses more than the

recommended 1 µL/bee dose for topical applications (being 2 µL/bee), this is satisfactorily justified in the full study report, and as the study validity criteria are met, this increase in topical dose is not considered to be a significant deviation.

The agreed endpoint considered suitable for use in the risk assessment is:

48 h LD_{50} (contact) = >100 µg a.s./bee

Report:	B.9.3.1/4 Amsel K., 2015a Acute toxicity of BAS 750 F to the bumblebee *Bombus terrestris* L. under laboratory conditions 2014/1275250 (Project no. 15 10 48 037 B)
Guidelines:	OECD 213 (1998), OECD 214 (1998), Hanewald et al. (2013), Van der Steen (1996), Van der Steen (2001)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no.: COD-001740; analysed purity: 98.8%.

B. STUDY DESIGN

Test species: *Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected on the morning prior to use. The bees were allowed to acclimatise to test conditions for 1 hour prior to application of the treatment.

Test design: In a 96-hour test, adults of *Bombus terrestris* were exposed to 5 doses of BAS 750 F in an appropriate carrier (acetone) placed on the dorsal bumblebee thorax. In total, 3 treatment groups were set up: 5 dose rates of the test substance, 3 control groups and 4 dose rates of the reference item with 30 replicates per dose and 1 bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were conducted after 4, 24, 48, 72 and 96 hours.

Endpoints: Mortality, behavioural impairments.

Reference item: BAS 152 11 I (dimethoate, nominal 400.0 g/L).

Test doses: Water control (deionised water), TritonX control (1% (v/v) TritonX solution), acetone control (pure acetone); reference item at dose rates of 2.5, 4.0, 6.3 and 10.0 µg dimethoate/bumblebee; test substance at dose rates of 12.5, 25.0, 50.0, 100.0 and 200.0 µg BAS 750 F/bumblebee. The test concentrations of BAS 750 F were prepared by serial dilution of a stock solution of the highest test concentration used, using pure acetone as the vehicle. The reference item was prepared in a similar fashion using 1 % v/v TritonX solution as the carrier. Before application, bumblebees were anaesthetised with CO_2 for approximately 20 seconds. Each bumblebee received a 4 µL dose of treatment solution to the dorsal thorax, using an Eppendorf Micropipette.

Test conditions:	Temperature: 24.6 °C – 25.2 °C, relative humidity: 58.8% – 60.9%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution. The bees were kept individually in Nicot cages (7 x 2 cm height x diameter) for the duration of the study. Food (50 % w/v sucrose solution) was supplied *ad libitum* for the duration of the study.
Statistics:	Descriptive statistics. Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$). Probit analysis using linear weight regression for calculation of the LD_{50} values for the reference item. Statistical analysis was carried out using the computer program ToxRat Professional 3.0 beta (2014).

II. RESULTS AND DISCUSSION

After 96 hours of contact exposure, no mortality occurred in the control groups treated neither with deionized water, TritonX solution nor with acetone. In the test substance treatment, no statistical significant mortality occurred after thoracic application of 12.5, 25.0, 50.0, 100.0 and 200.0 µg BAS 750 F/bumblebee, after 96 hours. The dose rate of 50.0 µg BAS 750 F/bumblebee revealed a slight mortality of 3.3%, which is not statistically significant when compared with the acetone control. Furthermore, no behavioural abnormalities of surviving bumblebees occurred throughout the contact toxicity test. The results are summarized in Table B.9.3.1/4-1.

Table B.9.3.1/4-1: Toxicity of BAS 750 F to *Bombus terrestris* (bumblebee) in a contact toxicity test

Treatment	Dosage	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	Water control	0.0	0.0	0.0	0.0
	1% TritonX [a]	0.0	0.0	0.0	0.0
	Acetone	0.0	0.0	0.0	0.0
BAS 750 F [µg a.s./bumblebee]	12.5	0.0	0.0	0.0	0.0
	25.0	0.0	0.0	0.0	0.0
	50.0	3.3	3.3	3.3	3.3
	100.0	0.0	0.0	0.0	0.0
	200.0	0.0	0.0	0.0	0.0
Reference Item [µg dimethoate/bumblebee]	2.5	0.0	0.0	0.0	0.0
	4.0	40*	40*	46.7*	46.7*
	6.3	50*	63.3*	63.3*	66.7*
	10.0	100*	100*	100*	100*
Endpoint [µg a.s./bumblebee]					
LD_{50} (96 h)	> 200.0				
Reference Item LD_{50} (96 h)	4.8 (48 h LD_{50} = 4.9)				

[a] 1% TritonX control belongs to reference item.

*Significant difference in pairwise comparison between treatment and respective control (Fishers Exact Binomial Test with Bonferroni Correction; $\alpha = 0.05$; one-sided greater).

III. CONCLUSION

In an acute contact toxicity study with BAS 750 F on bumblebees, the LD_{50} value (96 h) was estimated to be > 200.0 µg BAS 750 F/bumblebee.

RMS Comments

The study was carried out according to GLP. Currently no official guideline for acute toxicity of an active substance/formulation to bumblebees is in circulation, therefore the HSE evaluator has made reference to (yet to be noted) EFSA guidance, as well as the supplemental papers referred to by the applicant, in assessing the study. With reference to available guidance, no significant deviations were noted. No guidance was available regarding the appropriate sensitivity range for the reference item Dimethoate, although research by Hanewald *et al.* (2014) indicate an acute contact LD_{50} (96 h) of approximately 1.2-7.5 µg/bee (mean 5 µg/bee). The study report states an appropriate range for the contact test as between 10.0 and 2.50 µg a.s./bee, however as this range is not referenced there is a degree of uncertainty with it. The 96 h LD_{50} falls within this range (being 4.8 µg a.s./bee), and is close to the mean contact LD_{50} derived from the Hanewald *et al.* (2014) paper. This suggests that this batch of bumblebees displays average sensitivity. No mortality occurred in the controls, which meets the

validity criteria in OECD 214 (<10 % average control mortality). On balance the RMS considers the study to be reliable.

The agreed endpoint is:

96 h LD_{50} (oral) = >200.0 µg a.s./bumblebee

Report:	B.9.3.1/5 Kleebaum K., 2015a Chronic toxicity of BAS 750 F (Reg.No. 5834378) to the honeybee *Apis mellifera* L. under laboratory conditions 2013/1235086
Guidelines:	Decourtye *et al.* (2005), Suchail *et al.* (2001), CEB No. 230 (2012), Current ring test protocol of the AG-Bienenschutz (2014)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no.: COD-001740; analysed purity: 98.8% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: *Apis mellifera iberica* L. (honeybee); 1-4 day old bees; derived from a healthy and queen-right colony; source: Beekeeper Joaquin Cordero, Cazalla, Spain. The bees were hatched from 'five comb hive bodies' kept under test conditions until 1 day prior to the test start. Afterwards the newly hatched worker bees were transferred to their test cages in groups of 20/replicate, and were given 24 hours to acclimatise to test conditions.

Test design: In a 10-day test, young adults of *Apis mellifera* L. were exposed daily to 5 doses of BAS 750 F in treated food (50% w/v aqueous sucrose solution + 1 % v/v Tween20). In total, 4 treatment groups were set up: 5 doses of the test substance, 2 untreated controls and 4 doses of the reference item with 3 replicates per dose and 20 bees per replicate. Assessments of bee mortality and behavioural effects were conducted daily during the study.

Endpoints: Mortality, behavioural impairments.

Reference item: Dimethoate 400 EC (analysed content of a.s.: 400.9 g/L).

Test doses: Control 1: untreated diet (50% (w/v) aqueous sucrose solution)
Control 2: untreated diet (50% (w/v) aqueous sucrose solution with 1% Tween20)

Test substance treatments:

Nominal dose/concentration		Actual intake of test substance
Doses [µg a.s./bee]	**Concentrations [g a.s./kg food]**	**[µg a.s./be/day]**
6.2	0.160	8.3
12.5	0.320	13.3
24.9	0.641	26.9
49.9	1.281	48.2
99.8	2.562	110.5

Reference item treatments: 1.1, 2.2, 4.4 and 8.8 µg dimethoate/bee/day.
The test substance and control treatments were prepared daily. The range of test substance concentrations were prepared by step-wise serial dilution of a stock solution of the highest test dose (100.98 µg a.s./bee) with 50 % sucrose solution containing 1 % Tween20. The reference item doses were similarly prepared but only once before the test began (dimethoate being stable for 10 days when refrigerated). Each bee received a 33 µL dose of treatment solution per day. The treatment solutions were replaced daily.
The amount of food consumed was measured daily by comparing the weight of each syringe containing treatment solution before application and after removal from the test units.

Test conditions: Temperature: 33.3° C-35.0° C ; relative humidity: 46%-60%, photoperiod: 24 h darkness; food: 50% (w/v) aqueous sucrose solution. Test units were aluminium cages (20x15x10 cm) with holes in the lateral walls for ventilation and two glass plates (front and back) for observation.

Statistics: Descriptive statistics; for mortality data Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$). The median lethal doses/concentrations of test and reference item were calculated with Probit analysis using linear maximum likelihood regression.

II. RESULTS AND DISCUSSION

After 10 days of continuous exposure, a mean mortality of 1.7% in the detergent control and 1.7% in the control were observed. In the test substance group mortalities between 0.0 and 8.3% occurred, which were not statistically significantly increased compared to the control groups (Fishers Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). As no effects were observed, all LC_x (LD_x) values are greater than the highest test concentration (dose).

During the testing period no behavioural abnormalities could be observed in any test substance group. The results are summarized in Table B.9.3.1/5-1.

Table B.9.3.1/5-1: Cumulative mortality and toxicity endpoints of honeybees (*Apis mellifera* L.) exposed to BAS 750 F in a chronic oral toxicity test

Treatment [BAS 750 F]			**Mortality after 10 days**	
Actual daily mean doses [µg consumed a.s./bee/day]	**Overall doses [µg a.s./bee/day]**	**Concentration [g a.s./kg food]**	**Cumulative mortality [%]**	**Corrected cumulative mortality [%]**
Control	Control	Control	1.7	--
Tween control	Tween control	Tween control	1.7	--
8.3	6.2	0.160	6.7	5.1
13.3	12.5	0.320	8.3	6.7
26.9	24.9	0.641	1.7	0.0
48.2	49.9	1.281	3.3	1.6
110.5	99.8	2.562	0.0	0.0
Reference Item (Dimethoate 400 EC)			**Mortality after 10 days**	
Actual daily mean doses [ng consumed a.s./bee/day]	**Dosage of a.s. (ng/bee/day)**	**Concentration of a.s. (mg/kg diet)**	**Cumulative mortality [%]**	**Corrected cumulative mortality [%]**
4.7	5.902	0.152	1.7	0.0
8.4	9.837	0.253	15.0*	13.6
11.2	16.395	0.421	40.0*	39.0
24.0	27.326	0.702	95.0*	94.9

Endpoints		**10 days**
Test substance doses [µg consumed a.s./bee/day]	**LD_{50}**	> 110.5
	NOED [1)]	≥ 110.5
Test substance concentrations [g a.s./kg food]	**LC_{50}**	> 2.562
	NOEC [1)]	≥ 2.562

1) Statistically significant difference in pairwise comparison between treatment and untreated control-Fisher's Exact Binominal Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$).

In the reference item treatment, the LD_{50} was determined to be 12.7 ng consumed dimethoate/bee/day, which corresponds to an LC_{50} of 0.423 mg dimethoate/kg food.

III. CONCLUSION

In a 10 day chronic toxicity feeding test with BAS 750 F the NOED was determined to be ≥ 110.5 µg consumed a.s./bee/day, and the NOEC ≥ 2.562 g a.s./kg food, respectively. The LD_{50} and LC_{50} were determined to be > 110.5 µg consumed a.s./bee/day and > 2.562 g a.s./kg food.

RMS Comments

The study was carried out according to GLP. No official guidelines are currently available for chronic toxicity studies on Honeybees, therefore in evaluating this study reference has been made to previous

literature (as stated by the study authors), as well as the yet to be noted EFSA guidance (EFSA Journal 2013; 11(7): 3295), and the paper 'Proposal for a new OECD guideline for the testing of chemicals on adult honey bees (Apis mellifera L.) in a 10 day chronic feeding test in the laboratory and results of the recent ring test 2014' (Kling and Schmitzer, 2014). The study follows the guidance available with no deviations noted. The LC_{50} of the reference item (0.423 mg dimethoate/kg food) is close to that reported by Kling and Schmitzer (2014) (0.48 ± 0.15 mg dimethoate/kg food), and the mortality in both controls was less than 15% (being 1.7 % in both cases). Therefore the study meets the proposed validity criteria for a chronic honeybee toxicity study. Overall the RMS considers the study to be reliable.

The agreed endpoints are:

- **10 day LC_{50} = >2.562 g a.s./kg food (equivalent to >110.5 µg a.s./bee/day)**
- **10 day NOEC = ≥2.562 g a.s./kg food (equivalent to >110.5 µg a.s./bee/day)**

Report: B.9.3.1/6
Kleebaum K., 2015b
Acute toxicity of BAS 750 F to honeybee larvae (*Apis mellifera* L.) under laboratory conditions (in vitro)
2013/1235087

Guidelines: OECD 237 (2013) Honey bee (*Apis mellifera*) larval toxicity test single exposure

GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F; batch no.: COD-001740; analysed purity: 98.8% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: *Apis mellifera* L. subspecies *carnica* P. (honeybee); synchronized first instar larvae (one day old); derived from three healthy and queen-right colonies; source: Bienenfarm Kern GmbH, Leipzig, Germany. On day -3 (D-3) the respective queen of each colony was caged on an empty brood comb for approximately 30 hours. On D-2 the queen was released and the brood comb was kept excluded to prevent further egg-laying. On D1 the combs containing larvae were transported (in an insulated box for grafting) to an acclimatised laboratory room.

Test design: One day old honeybee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. At D4 larvae were exposed to a single application of BAS 750 F diluted in the larvae food (aqueous sugar solution mixed with royal jelly) for 96 hours (days test duration). In total, 4 treatment groups were set up: 5 doses of the test substance, 2 untreated control groups and 4 doses of the reference item with 3 replicates per dose and 12 larvae per replicate. After the day of application, additional feeding of the larvae took place 24 and 48 hours later. Assessments of larval mortality were done after 24, 48, 72 and 96 hours. Additionally, other observations such as small body size or large quantities of

remaining food after 72 (7 d test duration) and 96 hours (8 d test duration) were noted.

Endpoints: Mortality (NOED, LD_{50}), quantitative observations: body size, remaining food.

Reference item: Dimethoate technical (analysed purity: 99.8%).

Test doses: Control 1: untreated diet (50% aqueous sugar solution with 50% royal jelly)
Control 2: untreated diet with Tween20 and acetone (each 1% v/v)

Test substance treatments:

Nominal dose/concentration of BAS 750 F	
Doses [µg a.s./larva]	**Concentrations [g a.s./kg food]**
7.4	0.219
14.8	0.438
29.7	0.875
59.3	1.751
118.7	3.501

Five test substance solutions were prepared by serial dilution (with 50 % sucrose solution containing 1% v/v each of Tween20 and acetone) of a stock solution of 7.98 µg/µL. This initial stock solution's concentration was analysed using the HPLC method (LOQ = 68.65 mg a.s./L) and the mean recovery level was 106 %, supporting the use of nominal test concentrations. The solutions were diluted further with appropriate 2.0 g royal jelly to make each required test concentration. Each larva received a 30 µL dose.

Reference item treatments: 1.1, 2.2, 4.4 and 8.8 µg dimethoate/larva.

Test conditions: Temperature: 34.0° C – 34.5° C; relative humidity: 93%-97%, photoperiod: 24 h darkness; food: 50% aqueous sugar solution with 50% royal jelly. Diet A (D1) sucrose solution contained 12 % Glucose, 12 % Fructose and 2 % yeast w/v. Diet B (D3) contained 15 % Glucose, 15 % Fructose and 3 % yeast w/v. Diet C (D4, D5 and D6) contained 18 % glucose, 18 % fructose and 4 % yeast w/v.

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$) and No Observed Effect Level. The median lethal doses/concentrations of test and reference item were calculated with Probit analysis. Statistical analysis was carried out with ToxRat Professional 2.10.06 (2010).

II. RESULTS AND DISCUSSION

Control mortality was 2.8% and 13.9% after 72 hours and 96 hours of exposure, respectively (corresponding to a test duration of 7 and 8 days). The solvent control showed a mortality of 13.9% after 72 h which did not increase at 96 h of exposure. After 72 hours, larvae fed with 59.3 and 118.7 µg a.s./larva revealed a mortality of 91.7% and 83.3%, respectively, which was statistically significant in comparison to the solvent control group. The increase in mortality compared to the solvent control was statistically significant after 96 hours in these test substance groups as well, being 97.2% and 94.4% in the 59.3 and 118.7 µg a.s./larva treatment groups, respectively. The 96h (8d)

NOED was 29.7 µg a.s./larva, the calculated LD_{10} and LD_{50} were 22.1 and 43.9 µg a.s./larva, respectively.

After 72 hours of exposure (7 d test duration), reduced food intake occurred in 11.1%, 18.8%, 100.0% and 100.0% of the remaining individuals treated with 14.8, 29.7, 59.3 and 118.7 µg a.s./larva, respectively. After 96 hours (8 d test duration) of exposure, reduced food intake (and corresponding deviations to developing into an average sized larva) were still present in 3.3, 4.2, 100.0 and 50.0% of the remaining larvae, which were treated with 14.8, 29.7, 59.3 and 118.7 µg a.s., respectively. The results are summarized in Table B.9.3.1/6-1.

Table B.9.3.1/6-1: Toxicity of BAS 750 F to *Apis mellifera* (honeybee) in an acute oral larval toxicity test after exposure of 72 and 96 hours (7d and 8d test duration, respectively)

Dosage [µg a.s./larva]	Concentration [g a.s./kg food]	72 h mortality [%]		96 h mortality [%]	
		absolute	corrected [1)]	absolute	corrected [1)]
Control	Control	2.8	--	13.9	--
Tween control	Tween control	13.9	--	13.9	--
7.4	0.219	5.6	0.0	13.9	0.0
14.8	0.438	0.0	0.0	16.7	3.2
29.7	0.875	11.1	0.0	25.0	12.9
59.3	1.751	91.7 *	90.3	97.2 *	96.8
118.7	3.501	83.3 *	80.6	94.4 *	93.5
Reference Item (Dimethoate)					
1.1	0.032	25	22.9		
2.2	0.065	41.7*	40.0		
4.4	0.130	44.4*	42.9		
8.8	0.259	61.1*	60.0		
		Endpoints [µg BAS 750 F/bee]			
		72 h		96 h	
LD_{50} [µg a.s./larva] (95% confidence limits)		n.d.		43.9 (24.0 – 80.2)	
NOED [µg a.s./larva]		29.7		29.7	
LC_{50} [g a.s./kg food] (95% confidence limits)		n.d.		1.295 (0.710 – 2.363)	
NOEC [µg a.s./kg food]		0.875		0.875	

* Statistically significantly different compared to the control (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater; $\alpha = 0.05$).

1) According to Schneider-Orelli (1947).

In the reference item treatment group, larvae fed with 8.8 µg a.s./larva resulted in a mortality of 61.1% (corrected for control mortality: 60.0%), 72 hours after application. The LD_{50} value was determined to be 5.2 µg dimethoate/larva.

Validity Criteria
The study meets all the validity criteria specified in OECD 237:

- The mean mortality across both controls was less than 15 % (being 13.9 % in both controls after 96 h). However it was noted that in the solvent control, mortality exceeded 15 % in two of the replicates (both 16.7 %) after 72 hours – guidance stipulates that control mortality should be ≤ 15 % across replicates.
- The reference item 72 h LD_{50} was greater than 50 % at the dosage of 8.8 µg a.s./larva (being 60 % (corrected)), which meets the guideline recommendations for sensitivity.

III. CONCLUSION

In an acute oral larval toxicity study with BAS 750 F on honeybee larvae, the LD_{50} value (96 h exposure = 8 d test duration) was determined to be 43.9 µg a.s./larva (equivalent to LC_{50} = 1.295 g a.s./kg food). The NOED was determined to be 29.7 µg a.s./larva (equivalent to NOEC = 0.875 g a.s./kg food).

RMS Comments
The study was carried out according to GLP and follows the guideline OECD 237 with no significant deviations. The method of analysis of the active substance concentration in Diet C was confirmed as valid (III CA B.5.1.2.6) It was noted that the mortality in two of the solvent control replicates was greater than 15 % (being 16.7 %) after 72 hours; the non-solvent control mortality mirrored this after 96 hours. The RMS evaluator considered this to be an acceptable deviation as a) the exceedance of the limit was only by 1.7 %, b) the mean mortality was ≤ 15 %, and c) the larval grafting procedure integral to the assay is thought to make reliably maintaining low levels of mortality in the controls a difficult task. Therefore the study is considered to be valid.

The agreed endpoint is:

- **96 hr LD_{50} = 43.9 µg a.s./larva**

Report:	B.9.3.1/7 Royer S., 2015a Honey bee larvae test (repeated exposure, observation 21 days) under laboratory conditions (in vitro) 2014/1327676
Guidelines:	OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure; OECD Draft 'Guidance Document for honeybees larval toxicity test, repeated exposure' (2014)
GLP:	No

I. MATERIALS AND METHODS

A. MATERIALS

Test substance: BAS 750 F; batch no.: COD-001740; analysed purity: 98.8%.

B. STUDY DESIGN

Test species: Larvae of *Apis mellifera* L. subspecies *carnica* P. (honeybee); synchronized

first larval stage (L1); derived from three healthy and queen-right colonies; source: in-house colonies.

The larvae were selected from combs over which the colony queen had been confined in an excluder cage for approximately 30 hours. The combs were then left for 3 days within the cages, before transfer to the laboratory on day 1 of the study (D1). 32 larvae were selected from one colony and eight each from the other two colonies, per treatment group. Thus, there were 48 larvae per treatment group.

Test design: L1 honeybee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates. After this, in a 21 day chronic test, the larvae were fed during larval development with artificial diet, containing the test substance on rearing days 3, 4, 5 and 6. In total, 3 treatment groups were set up: 4 doses of the test substance, 1 untreated control group and 1 solvent control, each with 41 larvae from 3 different bee colonies. Survival was assessed over a time period of 21 days. Successful adult emergence was assessed on rearing day 21.

Endpoints: Mortality (21 day NOEC/NOED, 21 day LC_{50}/LD_{50}).

Test doses: Control 1: untreated diet (50% aqueous sugar solution with 50% royal jelly).

Control 2: untreated diet with acetone (0.5% w/w)
Test substance treatments:

Nominal dose/concentration of BAS 750 F	
Doses [µg a.s./larva]	**Concentrations [mg a.s./kg food]**
6.3	40.63
12.5	81.25
25.0	162.5
50.1	325.0

Diet A (D1) aqueous sugar solution contained 6 % each of glucose and fructose and 1 % Yeast extract, w/v. Diet B (D3) aqueous sugar solution contained 7.5 % each of glucose and fructose and 1.5 % Yeast extract, w/v. Diet C (D4-6) aqueous sugar solution contained 9 % each of glucose and fructose and 2 % Yeast extract, w/v.
On days 3 – 6, the well cellular culture plates were weighed immediately before and directly after feeding so that the actual fed amount of diet per plate could be calculated.
The test substance solutions were prepared daily during days 3-6 by serial dilution of a stock solution of the highest test concentration (325 mg a.s./kg diet).

Test conditions: Measured mean temperature and humidity:
34.7°C and 94.1% (days 1 – 8)
34.9°C and 78.3% (days 8 – 14)
35.7°C and 52.8% (days 14 – 21)

Statistics: Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) and No Observed Effect Level. Statistical analysis was carried out using ToxRatPro version 2.10.

II. RESULTS AND DISCUSSION

After 21 days, feeding of BAS 750 F in concentrations of 40.63, 81.25, 162.5 and 325 mg a.s./kg diet (corresponding to total doses of 6.3, 12.5, 25.0 and 50.1 µg a.s./larva) caused mean mortalities of 14.6%, 22.0%, 14.6% and 43.9%, respectively. These resulted in mean corrected mortalities of -20.7%, -10.3%, -20.7% and 20.7%, respectively. The mortality in the different treatments did not show statistically significant effects compared to the solvent control (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$).

At the end of 21 days, 75.6% and 70.7% of larvae honeybees emerged as adults in the untreated control and solvent control, respectively. In the test substance treatment groups, 85.4%, 78.0%, 85.4% and 56.1% emerged in the 6.3, 12.5, 25.0 and 50.1 µg a.s./larva test substance treatment groups, respectively. The results were not statistically significant compared to the solvent control. As no effects were observed, all LC_x (LD_x) values are greater than the highest test concentration (dose). The results are summarised in Table B.9.3.1/7-1.
It was also observed that 53 % of the surviving larvae treated with the test dose of 325 mg a.s./kg diet showed the presence of uneaten food at D8. This compares with 3, 0 and 7 % at the test concentrations of 40.63, 81.25 and 162.5 mg a.s./kg diet respectively, and 21 and 14 % in the control and solvent control, respectively.

Table B.9.3.1/7-1: Toxicity of BAS 750 F to *Apis mellifera* (honeybee) in a chronic oral larval toxicity test after 21 days

Dosage [µg a.s./larva]	**Concentration [mg a.s./kg food]**	**21 day mortality [%]**		**21 day adult emergence [%]** [2)]
		absolute	**corrected** [1)]	
Control	Control	24.4	--	75.6
Acetone solvent control	Acetone solvent control	29.3	--	70.7
6.3	40.63	14.6	-20.7	85.4
12.5	81.25	22.0	-10.3	78.0
25.0	162.5	14.6	-20.7	85.4
50.1	325.0	43.9	20.7	56.1
Endpoints [21 d]				
LD_{50} [µg a.s./larva]		> 50.1		
NOED [µg a.s./larva]		≥ 50.1		
LC_{50} [mg a.s./kg food]		> 325		
NOEC [mg a.s./kg food]		≥ 325		

1) Corrected for solvent control mortality according to Schneider-Orelli (1947).
2) Adult emergence is calculated as the reverse of the pupae mortality on day 21: adult emergence=100%-mortality (day21).

Validity Criteria
The study does not meet all of the study criteria proposed in OECD Draft Guidance:

- It is not clear whether the cumulative larval mortality was more than 15 % across replicates from D4-D7 (being 7.3 % in the control and 9.756 % in the solvent control on D5, but 17.07 % and 26.83 % respectively on D11 – no assessment was carried out on D7)
- Adult emergence in the controls was not less than 70 % (being minimum 70.7 %)

- The study did not include a reference item (Dimethoate) – therefore the sensitivity of the test system cannot be confirmed.

III. CONCLUSION

In a chronic oral larval toxicity study with BAS 750 F on honeybee larvae, the NOEC value (21 d) was determined to be ≥ 325 mg a.s./kg food. The NOED was determined to be ≥ 50.1 µg a.s./larva.

RMS Comments
The study was not carried out according to GLP, and according to Regulation EC 283/2013 GLP is a requirement for all studies to be used in risk assessments. The study follows the OECD 'Draft Guidance document for Honey Bee larval toxicity test, repeated exposure', with a number of deviations that add an element of uncertainty to the results derived.
Firstly it was noted that no reference item was tested alongside the test substance. This means that the sensitivity of the test system cannot be confirmed, and also that the study does not meet the drafted validity criteria.
Secondly, it was noted that fewer than 12 larvae were selected from two of the colonies (8 each), whilst 32 larvae were selected from the other colony. Guidance states that a minimum of 12 larvae from each colony should be selected. That this condition has not been met reduces the statistical power of the findings.
The apparently repellent effect of the test substance at the concentration of 325 mg a.s./kg diet is noted.

Overall given the lack of GLP certification, and the failure to meet the study validity criteria in draft guidance, the RMS evaluator does not consider the study to be suitable for risk assessment.

B.9.3.2. Effects on non-target arthropods other than bees

Summaries of the studies submitted in support of this application with regard to toxicity of the active substance to non-target arthropods are included in Section B.9.5.2 of Volume 3CP (DAR). The studies submitted test the formulation BAS 750 01 F, but they are used to address the risk from both the active substance BAS 750 F and the formulation. A summary of the available endpoints is presented in the table below.

Reference	**Organism**	**Study Type**	**LR_{50} (ml/ha)**	**ER_{50} (reproduction, ml/ha)**
Fallowfield L. 2015a	*T. pyri*	Tier 1	769.1	none
Stevens J. 2015a	*A. rhopalosiphi*		95.4	none
Fallowfield L. 2015b	*T. pyri*	Extended laboratory	>3000	>3000
Stevens J. 2015b	*A. rhopalosiphi*		>3000	>3000
Vaughan R. 2015a	*C. carnea*		>3000	>3000

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

Organism	Test substance	Timescale (Test type)	Endpoint [a]	Toxicity value (mg a.s./kg dry soil)	Reference
Eisenia fetida	BAS 750 F	56 days	NOEC $_{corr}$ **EC_{10} $_{corr}$** $EC_{50\ corr}$	4 **2.65** >8	B.9.4.1/1 Friedrich S., 2013a
Eisenia fetida	BAS 750 F	14 days	$NOEC_{corr}$ $LC_{50\ corr}$	31.25* > 500*	B.9.7/1 Friedrich S., 2015a
Folsomia candida	BAS 750 F	28 days	$NOEC_{mortality,\ reproduction}$ LC_{50} EC_{50}	≥ 400 > 400 > 400	B.9.4.2/1 Friedrich S., 2013b
Hypoaspis aculeifer	BAS 750 F	14 days	$NOEC_{mortality,\ reproduction}$ LC_{50} EC_{50}	≥ 1000 > 1000 > 1000	B.9.4.2/2 Schulz L., 2014a
Hypoaspis aculeifer	1,2,4-triazole	14 days	NOEC reproduction LC_{50} EC_{20} EC_{10}	171.0 > 1000 241 190	B.9.4.2/3 Schulz L., 2014b
Soil micro-organisms	BAS 750 F	28 days (nitrogen transformation)	Effects on nitrogen transformation	<25 % effects at 2.53	B.9.5/1 Schulz L., 2015a
Soil micro-organisms	BAS 750 F	28 days (carbon transformation)	Effects on carbon transformation	<25 % effects at 2.53*	B.9.7/2 Schulz L., 2015b

* Study summary is presented as additional information in section B.9.7, as these studies are no longer required according to EU Commission Regulation No.283/2013.

B.9.4.1. Earthworms

Report: B.9.4.1/1
Friedrich S., 2013a
Sublethal toxicity of Reg.No. 5834378 (BAS 750 F) to the earthworm *Eisenia fetida* in artificial soil
2013/1235075
Guidelines: OECD 222 (2004)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance:	BAS 750 F (Reg. No. 5 834 378), batch no. COD-001740, purity: 98.8% (analysed, ± 1.0%).

B. STUDY DESIGN

Test species:	*Eisenia fetida*; adult worms with clitellum and weight of 389-539 mg per worm, approximately 4 months old; source: W. Neudorff GmbH KG followed by in-house culture.
Test design:	In a 56-day test, adults of *Eisenia fetida* were exposed to five concentrations of BAS 750 F in treated artificial soil according to OECD 222 (10% peat). In total, 6 treatment groups were set up (5 concentrations of the test substance and an untreated control group) with 4 replicates for the test substance treatments and 8 replicates for the control, 10 adult worms per replicate. The artificial soil was treated and filled into vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioural effects and weight change were carried out after 28 days of exposure. After an additional 28 days (56 days after application), effects on reproduction (number of juveniles) were assessed.
Endpoints:	Mortality, weight change, feeding activity, reproduction rate.
Reference item:	Nutdazim 50 Flow (Carbendazim SC 500). Tested in a separate study dated 22.11.2013, where the number of juveniles was reduced by 39 and 100 % at concentrations 5 and 10 mg f.p./dsw, respectively, when compared to the control. This confirms the sensitivity of the test system (guidance recommends significant effects to be observed between 1 and 5 mg a.s./kg dsw)
Test concentrations:	Control, 1, 2, 4, 8 and 16 mg BAS 750 F/kg dry soil. Exactly weighed amounts of the test substance were mixed with finely ground quartz sand, such that 10 g of the respective mixture contained the amount of test substance required for each test vessel. The treated sand was then mixed thoroughly with the artificial soil using a laboratory mixer.
Test conditions:	Artificial soil according to OECD 222 with 10% peat, 20 % kaolin clay, 0.5 % $CaCO_3$, 69.5 % quartz sand, mixed with deionised water using a laboratory mixer then added to plastic test vessels (16.5 x 12 x 6 cm) in portions of 810 g wet weight (equivalent to 600 g dry weight). pH 6.13-pH 6.20 at test initiation, pH 5.74 – pH 5.83 at test termination; water content 55.9 %-56.3 % of its maximum water holding capacity (WHC) at test initiation and 55.3 %-55.9 % of WHC at test termination, temperature: 18.0°C – 21.6 °C; photoperiod: 16 hours light : 8 hours dark, light intensity: 530 lux, feeding with 5 g air-dried horse manure, once before application then once weekly thereafter until the end of the mortality assessment (after 4 weeks), then once more after removal of the adults, at the start of the reproductive phase. Worms were acclimatised for 24 hours prior to test start in a separate batch of artificial soil.
Statistics:	Descriptive statistics – arithmetic mean and standard deviation of change in biomass, reproduction and mortality; EC_{10}, 20 and 50 values (number of juveniles) were calculated by Probit analysis (Finney 1971). Shapiro-Wilk's test and Levene's test were used to test the data for normality and

homogeneity of variance, respectively. Fisher's Exact Binominal test for mortality with Bonferroni correction ($\alpha = 0.05$, one-sided greater).Williams-t-test for weight change and reproduction data ($\alpha = 0.05$, one-sided smaller). Statistical software used was ToxRat Professional 2.10.06 (Ratte 2010).

II. RESULTS AND DISCUSSION

BAS 750 F did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was between 0.0% and 5.0% in the test substance treatments and 2.5% in the control group. The weight change of adult worms was between 30.1% and 37.5% in the test substance treatments and 36.3% in the control group.
In the control, a mean of 108.3 juveniles was counted. In the test substance treatment groups, mean numbers of juveniles between 81.0 and 119.0 were counted. The reproduction rate was significantly reduced compared to the control at 16 mg a.s./kg dry soil, the highest treatment rate tested (Williams-t-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all test substance treated groups was comparable to the control. The EC_{10} for reproduction was determined to be 5.3 mg a.s./kg. The main results are summarised Table B.9.4.1/1-1.

Table B.9.4.1/1-1: Effects of BAS 750 F on *Eisenia fetida* in a 56-day reproduction study

BAS 750 F [mg a.s./kg dry soil]	**Control**	**1**	**2**	**4**	**8**	**16**
Mortality (28 d) [%]	2.5	0.0	2.5	2.5	2.5	5.0
Weight change (28 d) [%]	36.3	34.6	37.5	36.5	34.1	30.1
Number of juveniles (56 d)	108.3	112.5	119.0	97.5	91.8	81.0*
Coefficient of Variation (reproduction, %)	15.7	20.3	14.0	15.0	16.0	20.5
Reproduction (56 d) [% of control]	100	103.9	109.9	90.1	84.8	74.8
Endpoints [mg a.s./kg dry soil]						
NOEC (day 28)	≥ 16					
NOEC (day 56)	8					
EC_{10} (day 56)	5.3					
EC_{50} (day 56)	> 16					

* Statistically significantly different compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

Validity Criteria
The study meets all of the validity criteria specified in OECD 222:

- Control mortality after 4 weeks was no more than 10 % (being 2.5 %)
- Control number of juveniles per replicate was not less than 30 (being ≥81)
- Control coefficient of variation of reproduction was not more than 30 % (being 15.7 %)

III. CONCLUSION

In a 56-day reproduction study with BAS 750 F no adverse effects on survival and biomass development were determined at concentrations up to and including 16 mg a.s./kg dry soil.

Statistically significant reduction in the number of juveniles of *Eisenia fetida* was determined at 16 mg a.s./kg dry soil. Therefore the NOEC for reproduction was 8 mg a.s./kg dry soil.

RMS Comments
The study was carried out according to GLP and follows the guideline OECD 222 with no deviations noted.

The agreed endpoints considered suitable for use in the risk assessment are:

- **56 day EC_{10} (reproduction) = 5.3 mg a.s./kg dsw (3.266-8.712 95 % confidence interval)**
- **56 day NOEC (reproduction) = 8 mg a.s./kg dsw**

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Report:	B.9.4.2/1 Friedrich S., 2013b Effects of BAS 750 F on the reproduction of the collembolan *Folsomia candida* 2013/1235081
Guidelines:	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance:	BAS 750 F (Reg. No. 5 834 378), batch no. COD-001740, purity: 98.8% (analysed, ± 1.0%).

B. STUDY DESIGN

Test species:	Collembola (*Folsomia candida)*, juveniles (9-12 days old); source: in-house culture.
Test design:	28-day test in treated artificial soil according to ISO 11267 and OECD 232; artificial soil filled in glass vessels was treated with different concentrations of the test substance before collembolans were introduced on top of soil; 7 treatment groups (5 test substance concentrations, control, solvent control); 4 replicates for the test substance treatments, 8 replicates for the control and solvent control, each containing 10 juvenile collembolans. After 28 days the parental and juvenile collembolans were counted, following extraction by floatation in ink-darkened water. The efficiency of this extraction method was determined to be 97 % in a separate test.
Endpoints:	Mortality, behavioural effects, reproduction rate after 28 days.
Reference item:	Boric acid (100% analysed). The effects of the reference item were investigated in a separate study (July 2013). The EC_{50} (reproduction) was determined to be 108 mg a.s./kg dsw, which meets the OECD 232 recommendation for sensitivity of the test system (EC_{50} at approximately 100 mg a.s./kg dsw).

Test rates:	Controls (1 untreated, 1 acetone only), 25, 50, 100, 200 and 400 mg a.s./kg dry soil. The test substrates were prepared as follows. A stock solution of 0.405 g BAS 750 F in 10 mL acetone was prepared, which was then added directly to 10 g quartz sand (in the case of the highest test concentration), or diluted in appropriate quantities in 5 mL acetone prior to mixing with 10 g quartz sand (for the lower test concentrations). The acetone was then evaporated under a fume hood before adding 10 g of each mixture to 302.5 g soil (wet weight), and thoroughly mixed with a laboratory mixer.
Test conditions:	Artificial soil according to OECD 232 with a peat content of 5%, 20 % kaolin clay, 0.3 % $CaCO_3$, 74.7 % industrial quartz sand then (once treated) added to 150 mL capacity glass containers in 30 g portions per replicate (wet weight); pH 6.03 to pH 6.09 at test initiation, pH 5.76 – pH 5.84 at test termination; water content 56.8%-57.3% of maximum water holding capacity (WHC) at study initiation and 55.7%-56.6% of WHC at test termination; temperature: 19.6°C-20.7°C; photoperiod: 16 h light : 8 h dark, light intensity: 510 lux; food: 2 mg dry yeast at test start and after 14 days. Aeration twice per week.
Statistics:	Mortality (sum of missing and dead adults, %) was calculated for each treatment group. The reproductive output was calculated in comparison to the solvent control. For comparison with the solvent control-Fisher's Exact Binominal Test with Bonferroni correction for mortality data ($\alpha = 0.05$, one-sided greater), Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller). Statistical software used was ToxRat Professional 2.10.06 (Ratte 2010).

II. RESULTS AND DISCUSSION

In the test substance treatments mortality rates of 0% to 5% were observed, compared to 6.3% in the control and 5.0% in the solvent control. No statistically significant effect on mortality compared to the control was observed in any of the test substance concentrations. In the control a mean of 468 juveniles and in the solvent control 467 juveniles was counted. In the treatment groups a mean number of juveniles of 452 to 485 were counted. No statistically significant effects on the number of juveniles compared to the solvent control were recorded at any concentration tested. No differences between the behaviour of the collembolans in the control groups and the test substance groups could be observed. No EC_x value is presented as no clear dose response could be determined and no effects were observed at the highest rate. The results are summarized in Table B.9.4.2/1-1.

Table B.9.4.2/1-1: Effect of BAS 750 F on collembolans (*Folsomia candida*) in a 28-day reproduction study

BAS 750 F [mg a.s./kg dry soil]	**Control**	**Solvent control**	**25**	**50**	**100**	**200**	**400**
Mean Mortality (day 28) [%]	6.3	5.0	5.0	5.0	5.0	5.0	0.0
Mean No. of juveniles (day 28)	468	467	470	455	472	485	452
Coefficient of variation (Reproduction, %)	8.5	10.4	11.2	2.6	17.5	12.9	9.5
Mean Reproduction (day 28) [% of control]	--	100	101	98	101	104	97
Endpoints [mg a.s./kg dry soil]							
$NOEC_{mortality, reproduction}$	≥ 400						
LC_{50}	> 400						
EC_{50}	> 400						

Validity Criteria
The study meets all the validity criteria specified in OECD 232:

- Control mean mortality did not exceed 20 % at the end of the test (being 5.0-6.3 %)
- Control mean number of juveniles per vessel was at least 100 at the end of the test (being 467-468)
- Control coefficient of variation calculated for reproduction was less than 30 % at the end of the test (being 8.5 – 10.4 %)

III. CONCLUSION

In a 28-day collembolan reproduction study with BAS 750 F the EC_{50} was > 400 mg a.s./kg dry soil. The NOEC based on reproduction and mortality was ≥ 400 mg a.s./kg dry soil, the highest concentration tested.

RMS Comments
The study was carried out according to GLP and follows the guidelines specified in OECD 232/ISO 11267 with no significant deviations noted. It was noted that the spacing factor between test concentrations exceeded the recommended factor of 1.8 (being 2.0), however as no significant effects were observed in all of the test groups this is not thought to have had a significant impact on the results derived from the study. The study is considered to be reliable by the RMS.

The agreed endpoints considered suitable for use in the risk assessment are:

- **28 day NOEC (overall) = ≥ 400 mg a.s./kg dsw**
- **28 day L/EC_{50} = > 400 mg a.s./kg dsw**

Report: B.9.4.2/2
Schulz L., 2014a
Effects of BAS 750 F on the reproduction of the predatory mite *Hypoaspis aculeifer*
2013/1235082
Guidelines: OECD 226 (2008)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F batch no. COD-001740, purity: 98.8% (analysed, ± 1.0%).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult female predatory mites (age difference 3 days); source: in-house culture originally purchased from Katz Biotech AG, Baruth, Germany.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of BAS 750 F on mortality and reproduction of soil mites. Different concentrations of the test substance were homogenously mixed into artificial soil (5% peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 7 treatment groups (control, solvent control, 5 test substance concentrations); 8 replicates for the control treatments and 4 replicates for test substance treatments, each with 10 soil mites. After two weeks, the surviving mites and juveniles were extracted from each test unit using a MacFadyen high-gradient extractor and the levels of mortality and reproduction were assessed. Extraction efficiency was reported to be 95.5 % in a separate run.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400. The effects of the reference item were investigated in a separate study (Feb 2013). The EC_{50} of the reference item was calculated to be 6.64 mg a.s./kg dsw, which falls within the range described by guidance (3-7 mg a.s./kg dsw), thereby confirming the sensitivity of the test system.

Test rates: Untreated and solvent control (acetone), 62.5, 125, 250, 500 and 1000 mg test substance/kg dry soil. An exactly weighed amount of the test substance was dissolved in acetone to make a stock solution. This stock solution was stepwise diluted with acetone to prepare 4 further test solutions. Afterwards the test solutions were mixed with quartz sand, and the acetone evaporated under a fume hood for at least 1 hour. The test substance mixtures were then mixed with the artificial soil with a laboratory mixer.

Test conditions: Artificial soil according to OECD 226 – 5 % sphagnum peat, 20 % kaolin clay, 0.2 % $CaCO_3$, 74.6 % industrial quartz sand, mixed with deionised water in a laboratory mixer, then (once treated) added to 100 mL Schott-bottles with screw caps (4cm diameter) in 20 g dsw portions; pH 5.5 – pH 5.7 at test initiation, pH 5.7 – pH 5.8 at test termination; water content at test initiation 49.69% – 58.24% of maximum water holding capacity (WHC) and 48.28% – 57.30% of maximum WHC at test termination; temperature:

19.5°C – 21.1°C; photoperiod: 16 h light : 8 h dark; light intensity: 522 lux; food: cheese mites (*Tyrophagus putrescentiae*) supplied twice to three times a week (aeration took place at the same time as feeding).

Statistics: Mortality in % for each treatment group was calculated (missing mites counted as dead). Reproductive output for each treatment group (%) was compared to the solvent control; Fisher's Exact Binominal Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), Dunnett-t-test for reproduction ($\alpha = 0.05$, one-sided smaller). Statistical software: ToxRat Professional 2.10.05 (RATTE 2010).

II. RESULTS AND DISCUSSION

Test substance treatment groups had mortality rates of between 0.0%-7.5%. In the untreated control and the solvent control the mortality rate was 5.0% and 2.5%, respectively. The observed mortality rates for adult mites in the test substance treatment groups compared to the solvent control were not statistically significant. Differences between the behaviour and the morphology of the mites in the solvent control and the test substance treatment groups could not be observed.

In the untreated and the solvent control group, mean numbers of 332.6 and 309.4 juveniles were counted, respectively. In the test substance treatment groups the mean number of juveniles was between 293.0 and 330.8. BAS 750 F showed no statistically significantly adverse effects on reproduction at all test concentrations. No EC_x value is presented as no clear dose response could be determined and no effects were observed at the highest rate. The results are summarised in Table B.9.4.2/2-1.

Table B.9.4.2/2-1: Effects of BAS 750 F on predatory mite (*Hypoaspis aculeifer*) mortality and reproduction (day 14)

BAS 750 F [mg a.s./kg dry soil]	**Control**	**Solvent control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality [%]	5.0	2.5	7.5	2.5	0.0	0.0	0.0
No. of juveniles (day 14)	332.6	309.4	296.5	322.5	293.0	330.8	298.3
Coefficient of variation (reproduction; %)	14.6	16.7	11.0	3.2	22.5	6.8	13.6
Reproduction (day 14) [% of solvent control]	--	100	96	104	95	107	96
Endpoints [mg a.s./kg dry soil]							
$NOEC_{mortality + reproduction}$	≥ 1000						
LC_{50}	> 1000						
EC_{50}	> 1000						

Validity Criteria

The study meets the validity criteria specified in OECD 226:

- Control mortality did not exceed 20 % at the end of the test (being 2.5 – 5 %)
- Control mean number of juveniles per replicate was at least 50 at the end of the test (being 309.4 – 332.6)

- The coefficient of variation calculated for the number of juvenile mites per replicate in the control was not more than 30 % at the end of the test (being 14.6-16.7 %)

III. CONCLUSION

In a 14-day reproduction study with BAS 750 F on predatory soil mites (*Hypoaspis aculeifer*), the LC_{50} and EC_{50} values were determined to be > 1000 mg a.s./kg dry soil. The NOEC for mortality and reproduction was determined to be ≥ 1000 mg a.s./kg dry soil.

RMS Comments
The study was carried out according to GLP and follows the guidelines specified in OECD 226 with no significant deviations noted. It was noted that the spacing factor between test concentrations exceeded the recommended factor of 1.8 (being 2.0), however as no significant effects were observed in all of the test groups this is not thought to have had a significant impact on the results derived from the study. The study is considered reliable by the RMS.

The agreed endpoints considered suitable for use in the risk assessment are:

- **14 day NOEC (overall) = ≥ 1000 mg a.s./kg dsw**
- **14 day L/EC_{50} = > 1000 mg a.s./kg dsw**

Report: B.9.4.2/3
Schulz L., 2014b
1,2,4-triazole-CGA71019-Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
2014/1326895
Guidelines: OECD 226 (2008)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: CGA71019 (=1,2,4-triazole); batch no. R 200; analysed purity: 99.0% (tolerance ± 2.0%).

B. STUDY DESIGN

Test species: Soil mites: *Hypoaspis aculeifer* (CANESTRINI); adult females with an age difference of 2 days, reared in-house.

Test design: The effects of the test substance on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226. Different concentrations of the test substance were homogeneously mixed into the artificial soil (5% peat) which was then filled into glass vessels after which the soil mites were introduced on top of the soil; 10 treatment groups (9 test substance concentrations, control); 8 replicates/control group and 4 replicates/test substance treatment group each with 10 soil mites. After two weeks, the surviving mites and juveniles were extracted from each test unit using a MacFadyen high-gradient extractor and the levels of mortality and reproduction were assessed.

Endpoints:	Mortality and reproduction rate (no. juveniles) after 14 days.
Reference item:	Dimethoate EC 400 (content of a.s. dimethoate: 400 g/L nominal). The effects of the reference item were investigated in a separate study (February 2013). The EC_{50} of the reference item was calculated to be 6.64 mg a.s./kg dsw, which falls within the range described by guidance (3-7 mg a.s./kg dsw), thereby confirming the sensitivity of the test system.
Test concentrations:	Control (deionised water only) , 9.07, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test substance/kg dry soil. The test substance was mixed with finely quartz sand to prepare a stock mixture which was added directly to artificial soil to prepare the highest test concentration. The stock mixture was stepwise diluted with quartz sand to prepare 8 further test mixtures, which were added to artificial soil and mixed by means of a hand stirrer to prepare the remaining test concentrations.
Test conditions:	Artificial soil according to OECD 226-5 % sphagnum peat, 20 % kaolin clay, 0.2 % $CaCO_3$, 74.7 % industrial quartz sand, mixed with deionised water in a laboratory mixer, then (once treated) added to 100 mL Schott-bottles with screw caps (4cm diameter) in 20 g dsw portions. pH 6.0 at test initiation, pH 5.7-pH 6.0 at test termination; water content at test initiation 49.47%-51.83% of maximum water holding capacity (WHC) and 48.5%-51.52% of maximum WHC at test termination; temperature 19.5 °C – 21.2 °C; photoperiod: 16 h light : 8 h dark; light intensity: 511 lux. Feeding of mites with *Tyrophagus putrescentiae*, every 2-3 days, in combination with aeration of the test vessels.
Statistics:	The percentage mortality was calculated (missing mites counted as dead), and corrected for mortality in the control group using Abbott's formula (1925). Reduction of reproductive output was calculated for the treatment groups in comparison to the control; Fisher's Exact Binomial Test with Bonferroni Correction for mortality ($\alpha = 0.05$) and Williams-t-test for reproduction ($\alpha = 0.05$), Probit Analysis for EC-values. Statistical software: ToxRat Professional 2.10.05 (RATTE 2010).

II. RESULTS AND DISCUSSION

Adult soil mite mortality rates of 0.0% to 10.0% were recorded in the test substance treatment groups, compared to 3.8% mortality in the control group. This resulted in corrected mortality rates ranging from -1.3% to 6.5% in the treatment groups. The observed mortality rates in the test substance treatment groups compared to control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$).

The mean number of juveniles was 187.0 in the control and 20.0-244.8 in the groups treated with the test substance. The test substance caused no statistically significantly adverse effects on reproduction up to and including a test concentration of 171 mg test substance/kg dry soil. Statistically significant effects on reproduction could be observed at 309, 556 and 1000 mg test substance/kg dry soil (Williams-t-test, $\alpha = 0.05$). The results are summarised in Table B.9.4.2/3-1.

Table B.9.4.2/3-1: Effects of the test substance CGA71019 (=1,2,4-triazole) on *Hypoaspis aculeifer* in a 14-day reproduction study

CGA71019 [mg/kg dry soil]	**Control**	**9.07**	**16.3**	**29.4**	**52.9**	**95.3**	**171**	**309**	**556**	**1000**
Mortality (day 14) [%]	3.8	10.0	2.5	0.0	2.5	2.5	0.0	0.0	2.5	5.0
Corrected Mortality (%)	-	6.5	-1.3	-3.9	-1.3	-1.3	-3.9	-3.9	-1.3	1.3
Mean no. of juveniles (day 14)	187.0	212.8	200.3	203.5	239.0	244.8	184.0	116.3 *	41.8 *	20.0 *
Coefficient of Variation (Reproduction; %)	16.4	32.5	13.4	13.1	25.9	29.3	25.9	13.3	6.6	7.1
Reduction in Reproduction (day 14) [%]	--	-13.8	-7.1	-8.8	-27.8	-30.9	1.6	37.8	77.7	89.3
Endpoints [mg CGA71019/kg dry soil]										
$NOEC_{mortality}$	≥ 1000									
$NOEC_{reproduction}$	171.0									
LC_{50}	> 1000									
EC_{10}	190 mg test substance/kg dsw (95 % c.i. 158-229 mg metabolite/kg dsw)									
EC_{20}	241 mg test substance/kg dsw (95 % c.i. 210-276 mg metabolite/kg dsw)									

* Statistically significantly different compared to the control (William's t-test, $\alpha = 0.05$).
(Negative values indicate an increase in reproductive output)

Validity Criteria
The study meets the validity criteria specified in OECD 226

- Control mortality did not exceed 20 % at the end of the test (being 3.8 %)
- Control mean number of juveniles per replicate was at least 50 at the end of the test (being 187)
- The coefficient of variation calculated for the number of juvenile mites per replicate in the control was not more than 30 % at the end of the test (being 16.4 %)

III. CONCLUSION

In a 14-day reproduction study with the test substance CGA71019 (=1,2,4-triazole) on soil mites (*Hypoaspis aculeifer)*, the LC_{50} was estimated to be > 1000 mg test substance/kg dry soil. The NOEC for mortality was determined to be ≥ 1000 mg test substance/kg dry soil, while the NOEC for reproduction was determined to be 171 mg test substance/kg dry soil.

RMS Comments
The study was carried out according to GLP and follows the guidelines specified in OECD 226 with no deviations noted. The study is considered to be reliable by the RMS.

The agreed endpoints considered suitable for use in the risk assessment are:

- **14 day NOEC (reproduction) = 171 mg metabolite/ kg dsw**
- **14 day L/EC_{10} = 190 mg metabolite/kg dsw**

B.9.5. EFFECTS ON SOIL TRANSFORMATION

Report: B.9.5/1
Schulz L., 2015a
Effects of BAS 750 F (Reg.No. 5834378) on the activity of soil microflora (Nitrogen transformation test)
2015/1108623
Guidelines: OECD 216 (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch No. COD-001740, analysed purity: 98.8 % (tolerance ± 1.0%).

B. STUDY DESIGN

Soil Properties: Biologically active agricultural soil: loamy sand/sandy loam (USDA), 62.3 % sand, pH 6.4, 1.44 % C_{org} (microbial biomass 3.21 % of C_{org}), WHC: 35.59 g/100 g dry soil. The soil was carefully dried and sieved then stored at a temperature of approx. 4 °C under aerobic conditions. Before application the soil was adapted to test conditions for 7 days.

Test design: Determination of the N-transformation (NO_3-N-production) in soil enriched with lucerne meal (concentration in the soil 0.5%). Comparison of test substance treated soil with a non-treated soil. Sub-samples (3 replicates per treatment) were withdrawn from the bulk batches and subjected to the measurement. 10 g samples were taken at 3 hours, 7, 14 and 28 days after application. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process were determined using an Autoanalyzer (BRAN and LUEBBE).

Endpoints: Effects on the NO_3-N production 0, 7, 14 and 28 days after application.

Test concentrations: Control, 0.51 mg and 2.53 mg BAS 750 F/kg dry soil. 200g soil (dry weight, dsw) per test vessel (500 mL glass flask) was mixed with 0.5 % w/w Lucerne meal (1g/200 g dsw) by means of a hand-stirrer. The test substance was thoroughly mixed with quartz sand to make a stock mixture, which was further diluted with sand to create the test concentrations required. The subsequent mixtures were each mixed with prepared amounts of soil (10g/kg dsw) by means of a hand stirrer. Water was added to reach an actual water content of approx. 45 % of WHC.

Reference item: Dinoterb (purity: 98.0% ± 0.5% analysed). The reference item was tested in a separate study at rates of 6.80, 16.00 and 27.00 mg/kg.

Test conditions: Soil moisture: 45.5 – 48.31 % of maximum water holding capacity; measured water content: 16.19-17.19 g/100 g dry soil; pH 6.1-6.2. Soil samples were incubated at 19.4-20.8°C while stored in glass flasks in the dark.

Statistics: Mean nitrogen content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date. The relative deviations (%) between control and treatment values were calculated.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 750 F on nitrogen transformation in soil could be observed at both test concentrations (0.51 mg/kg dry soil and 2.53 mg/kg dry soil) after 28 days (time interval 0-28). Only negligible deviations from the control of -3.7% (test concentration 0.51 mg/kg dry soil) and +2.1% (test concentration 2.53 mg/kg dry soil) were measured at the end of the 28-day incubation period.

The results are summarised in Table B.9.5/1-1.

Table B.9.5/1-1: Effects of BAS 750 F on soil micro-organisms (nitrogen transformation) on days 0, 7, 14 and 28 days of incubation

Time interval (days)	Control	0.51 mg BAS 750 F per kg dry soil		2.53 mg BAS 750 F per kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
0-7	30.67	31.13	+1.5	31.67	+3.3
0-14	44.53	44.20	-0.7	45.03	+1.1
0-28	64.27	61.87	-3.7	65.60	+2.1

[1)] Based on NO_3-N production;-= inhibition, + = stimulation.

Validity Criteria

The study meets the validity criteria specified in OECD 216:

- The coefficient of variation between control replicate nitrate concentrations was less than 15 % (being maximum 5.1 %)

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of +33.2% and +46.9% at 16.00 mg and 27.00 mg/kg dry soil, respectively, determined 28 days after application.

III. CONCLUSION

Based on the results of this study, in accordance with OECD guideline 216, BAS 750 F caused no adverse effects (< 25% deviation from control) on the soil nitrogen transformation (measured as NO_3-N production) up to a concentration of 2.53 mg BAS 750 F/kg dry soil, after a 28-day incubation period.

RMS Comments
The study was carried out according to GLP and follows guideline OECD 216 with no deviations noted. The transformation rates were recalculated by the RMS, and the differences are still < 25 % for each period:

	Deviation from the control (%)		
	0-7	**7-14**	**14-28**
0.51 mg/kg	-1.52	5.77	11.49
2.53 mg/kg	-3.26	3.61	-4.22

The agreed endpoint considered suitable for use in the risk assessment is:

- **<25 % effects at 2.53 mg a.s./kg dsw, the maximum concentration tested**

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

No screening studies on non-target plants have been performed, and none are required.

B.9.6.2. Testing on non-target plants

Summaries of the studies submitted in support of this application with regard to toxicity to non-target plants are included in Section 9.11.2 of Volume 3CP (DAR). The seedling emergence and vegetative vigour studies, included below, test the formulation BAS 750 01 F, but they are used to address the risk from both the active substance BAS 750 F and the formulation.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

This section contains study data submitted in support of the application that is no longer a requirement for the risk assessment of the active substance according to Commission regulation EU no. 283/2013, and are summarised here to provide additional information.

Report:	B.9.7/1 Friedrich S., 2015a Acute toxicity of BAS 750 F to the earthworm *Eisenia fetida* in artificial soil with 10% peat 2015/1003342
Guidelines:	OECD 207 (1984), ISO 11268-1 (1993)
GLP:	Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, batch no. COD-001740, purity: 98.8% (analysed, ± 1.0%).

B. STUDY DESIGN

Test species:	*Eisenia fetida*; adult worms with clitellum and weight of 303-487 mg, age: approximately 3 months old; source: in-house culture. The worms were acclimatised for 24 hours prior to test start in untreated artificial soil.
Test design:	In a 14-day acute test, adults of *Eisenia fetida* were exposed to five concentrations of BAS 750 F in treated artificial soil according to OECD 207. In total, 6 treatment groups were set up (5 concentrations of the test substance and untreated control group) with 4 replicates, 10 adult worms per replicate. The artificial soil was treated and filled into 1 L capacity glass vessels (with a lid which allowed gaseous exchange; 751 g wet weight/replicate), before the earthworms were introduced on the top of the soil. Assessment of worm mortality was conducted 7 and 14 days after exposure, and biomass development and behavioural effects 14 days after exposure at test termination.
Endpoints:	Mortality, biomass development.
Reference item:	2-Chloroacetamide. In a separate study with the reference item, the 14-day LC_{50} was calculated as 21.3 mg a.s./kg dry soil with 95% confidence limits ranging from 20.3 to 22.4 mg a.s./kg dry soil. The test result is within the range described in ISO 11268-1 (1993)-20-80 mg/kg-indicating the test system is sufficiently sensitive.
Test concentrations:	Control, 62.5, 125, 250, 500, 1000 mg BAS 750 F/kg dry soil. Exactly weighed amounts of the test substance were mixed with finely ground quartz sand such that 10 g of the mixture contained the amount of test substance required for one replicate to adjust the selected concentration. The treated quartz sand (10 g per replicate) was mixed with 741 g artificial soil (wet weight) per replicate, and mixed using a laboratory mixer.
Test conditions:	Artificial soil according to OECD 207 (10% peat, 20 % Kaolin clay, 0.5 % calcium carbonate, 69.5 % quartz sand) pH 5.97-pH 6.12 at test initiation, pH 5.74 – pH 5.85 at test termination Water content 56.4%-56.7% of its maximum water holding capacity (WHC) at test initiation and 55.7%-56.1% of WHC at test termination Temperature: 19.1°C – 22.0°C Photoperiod: continuous illumination, light intensity: 530 lux.
Statistics:	Descriptive statistics; Williams-t-test for biomass data ($\alpha = 0.05$, one-sided greater). Software: ToxRat Professional 3.1.0 (2015).

II. RESULTS AND DISCUSSION

After 14 days of exposure, 0% mortality was observed in all test substance concentrations and in the control group. The biomass development was not statistically significantly different compared to the control at 62.5 mg BAS 750 F/kg dry soil, but was statistically significantly different at all other test concentrations (William's t-test, $\alpha = 0.05$, one-sided greater). No abnormal behaviour of the worms was observed in the test substance treatment groups or control group during the test. The results are summarised in Table B.9.7/1-1.

Table B.9.7/1-1: Effects of BAS 750 F on *Eisenia fetida* in a 14-day acute study

BAS 750 F [mg/kg dry soil]	Control	62.5	125	250	500	1000
Mortality (28 d) [%]	0	0	0	0	0	0
Biomass development (14 d) [%]	-6.1	-9.0	-11.6 *	-16.9 *	-21.5 *	-26.2 *
Endpoints [mg BAS 750/kg dry soil]						
LC_{50}	> 1000					
NOEC	62.5					

* Statistically significant difference compared to the control (Williams-t-test, $\alpha = 0.05$, one-sided greater).

Validity Criteria:
The study meets the validity criteria outlined in OECD 207/ISO 11268-1 (1993):
- Control mortality was less than 10 % (being 0 %)
- Loss of biomass in the control was no more than 20 % (being 6.1 %)

III. CONCLUSION

In a 14-day acute toxicity study with earthworms (*Eisenia fetida*), exposure to BAS 750 F resulted in an LC_{50} estimated to be greater than 1000 mg BAS 750 F/kg dry soil. The NOEC (biomass) was determined to be 62.5 mg BAS 750 F/kg dry soil.

RMS Comments:
The study was conducted according to GLP and follows the guidelines OECD 207/ ISO 11268-1 (1993) with no significant deviations noted. The quartz sand content of the artificial soil was lower than recommended in guidance (69.5 instead of 70 %), however this is a marginal difference and as the study validity criteria were fulfilled this is not considered a significant deviation from the guideline. Acute earthworm studies are not a requirement for assessing the risk to the soil compartment under regulation EC 1107/2009, but the data may provide useful additional information.

The agreed endpoints are:
14 d LC_{50} = >1000 mg a.s./kg dsw
14 d NOEC (biomass) = 62.5 mg a.s./kg dsw

Report: B.9.7/2
Schulz L., 2015b
Effects of BAS 750 F (Reg.No. 5834378) on the activity of soil microflora (Carbon transformation test)
2015/1108621
Guidelines: OECD 217 (2000)
GLP: Yes

I. MATERIAL AND METHODS

A. MATERIALS

Test substance: BAS 750 F, Batch No. COD-001740, analysed purity: 98.8 % (tolerance ± 1.0%).

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), pH 6.4, 1.44% C_{org}, (carbon content of microbial biomass = 3.21 % of C_{org}). WHC: 35.59 g/100 g dry soil. Removed to a depth of 20 cm from collection site (no fertilisers since 2003, last application of plant protection products 1990) prior to drying at room temperature and storage at 4 °C. Before test start the soil was adapted to test conditions for 1 week.

Test design: Comparison of test substance treated soil with a non-treated and a reference item treated soil. 3 replicates per concentration. A "BSB-digi" respirometer system was used to measure the O_2-consumption over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment. Sub-samples (100 g dsw per replicate) were withdrawn from the bulk batches and subjected to measurement. Glucose was then added such that a concentration of 0.6 % was attained, before filling into 500 mL reaction flasks. A smaller vessel containing 18 mL 1M NaOH was added. The reaction flasks were then tightly sealed and the cumulative oxygen production was measured over a period of 12 hours.

Test concentrations: Control, 0.51 mg and 2.53 mg BAS 750 F/kg dry soil. An exactly weighed amount of test substance was mixed with quartz sand to create a stock mixture, which was then diluted with quartz sand 500-fold and 100-fold such that 10 g of treated sand contained the amount of test substance required for one replicate to adjust the selected concentration. The treated quartz sand (10 g per replicate) was mixed with 1107.5 g artificial soil (wet weight) per replicate and mixed using a laboratory mixer.

Endpoints: Effects on O_2 consumption over 28 days of exposure.

Reference item: Dinoterb (purity: 98.0% ± 0.5% analysed). In a separate study (6th January-3rd February 2015) the reference item Dinoterb caused an inhibition of carbon transformation of-30.1% and -39.6% at 16.00 mg and 27.00 mg a.s./kg dry soil, respectively, determined 28 days after application.

Test unit: Each replicate was incubated in a 4 L steel vessel with lids that permitted gaseous exchange.

Test conditions: Soil moisture: 47.36-49.93% of its maximum water holding capacity: measured water content: 16.86-17.77 g/100 g dry soil. pH: 6.1-6.3.
Soil samples were incubated at 19.4-20.8 °C while stored in steel vessels in the dark.

Analysis: Cumulative oxygen consumption was calculated using regression analysis over 12 hours. Standard deviation and coefficient of variation were also calculated, as well as deviation from the control (%).

II. RESULTS AND DISCUSSION

No adverse effects of BAS 750 F on carbon transformation in soil could be observed at both test concentrations (0.51 mg/kg dry soil and 2.53 mg/kg dry soil) after 28 days. Only negligible deviations from the control of +1.1% (test concentration 0.51 mg/kg dry soil) and -1.1% (test concentration 2.53 mg/kg dry soil) were measured at the end of the 28-day incubation period. The results are summarised in Table B.9.7/2-1.

Table B.9.7/2-1: Effects of BAS 750 F on soil micro-organisms (carbon transformation) on days 0, 7, 14 and 28 of incubation

Soil (days)	Control		0.51 mg BAS 750 F/kg dry soil		2.53 mg BAS 750 F/kg dry soil	
	O_2 consumption [mg/h/kg dry soil]	Coefficient of Variation (%)	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from control [1)]
Loamy sand soil (0 d)	17.03	1.4	16.91	-0.7	17.22	+1.1
Loamy sand soil (7 d)	16.60	2.0	17.30	+4.2	16.57	-0.2
Loamy sand soil (14 d)	15.50	2.6	15.61	+0.7	15.37	-0.8
Loamy sand soil (28 d)	14.32	1.6	14.47	+1.1	14.16	-1.1

[1)] Based on O_2-consumption;-= inhibition; + = stimulation

Validity Criteria
The study fulfils the validity criteria described in OECD 217:

- Coefficient of variation in the controls not more than 15 % (being max 2.6 %)

III. CONCLUSION

Exposure of BAS 750 F in a field soil up to a test concentration of 2.53 mg BAS 750 F/kg dry soil caused no adverse effects (deviation from control < 25%, OECD 217) on the soil carbon transformation (measured as O_2-consumption) at the end of the 28-day incubation period.

RMS Comments
The study was carried out according to GLP and follows OECD guideline 217 with no deviations noted. Carbon transformation studies are not a requirement for assessing the risk to the soil compartment under regulation EC 1107/2009, but the data may provide useful additional information.

The agreed endpoint is:

<25 % adverse effects on carbon transformation at 2.53 mg a.s./kg dsw, the maximum concentration tested.

No additional studies that did not fit under the previous sections have been included as part of the submitted dossier.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Report:	B.9.8/1 Hammer S., 2014a BAS 750 F-Determination of the inhibition of oxygen consumption in the activated sludge respiration inhibition test 2014/1049095
Guidelines:	OECD 209 (2010)
GLP:	Yes

I. MATERIAL AND METHODS

Test substance:	BAS 750 F, batch no. COD-001740, purity: 98.8% (± 1.0% tolerance).
Test System:	Activated sludge from wastewater plants in Mannheim (Germany) treating municipal sewage. The suspension was sieved (mesh size 1mm) and aerated overnight at room temperature. The next day the suspension was washed once with drinking water and adjusted to 3 g/L (dry weight).
Test design:	Assessment of the inhibitory effect of the test substance on the oxygen consumption rate of aerobic micro-organisms (activated sludge, measurements took place over 8-10 mins) after short-term exposure of 3 hours; the inoculum was aerated during the contact period; 3 replicates for the test substance, 2 replicates for the reference item and 6 replicates for the control, final sludge concentration: 1.5 g/L dry weight.
Test concentrations:	Control, 1000, 500, 250, 125 and 62.5 mg a.s./L (without correction of purity or composition). The test substance was weighed in the required amounts for the test concentrations and added directly to the test vessels with approximately 234 mL deionised water. Control received 234 mL deionised water only. 16 mL synthetic medium was dosed to each test vessel followed by 250 mL inoculum suspension.
Reference item:	3.5-dichlorophenol. The reference item was applied at 1, 10 and 100 mg/L. The reference item was applied to the test system by adding appropriate quantities of a stock solution to each test vessel, followed by 234 mL deionised water, 250 mL inoculum suspension and 16 mL synthetic medium.
Test conditions:	Temperature: 21.1-21.3 °C; pH 7.5-7.6 (initial) 8.0-8.1 (end); 1 L glass beakers, 500 mL of test mixture per vessel.
Statistics:	Descriptive statistics, probit method for calculation of EC_x values. Software used was TOXRAT Professional 2.10.

II. RESULTS AND DISCUSSION

No significant inhibition of respiration was measured up to the highest tested concentration of 1000 mg a.s./L (nominal).

	Control	**Reference item [mg/L]**			**Test substance [mg/L]**				
		1	10	100	62.5	125	250	500	1000
O_2 consumption rate (mg/L x h; MEAN VALUES)	39.17	34	17	3.5	38	35.7	34.3	34.7	35.3
Inhibition of respiration compared to control (%)*	n/a	13	56.5	91	2.7	8.9	12.4	11.4	9.9

*****Based on mean values

Validity Criteria
The study meets the validity criteria specified in OECD 209:

- Control respiration rate coefficient of variation was no greater than 15 % (being 5.5 %)
- EC_{50} of 3,5-dichlorophenol was 7.6 mg/L, within the recommended range (5-30 mg/L)

III. CONCLUSION

The EC_{20} and EC_{50} values of BAS 750 F in the activated sludge respiration inhibition test are both > 1000 mg a.s./L. Disturbances in the bio-degradation process of activated sludge are not to be expected if the test substance is correctly introduced into adapted wastewater treatment plants at low concentrations.

RMS Comments
The study was carried out according to GLP and follows OECD 209 with no significant deviations. The preparation of the inoculum from municipal sewage deviated from guidance in that it was only washed once (instead of 3 times) and was suspended at 3 g/L dry weight (instead of 4 g/L dry weight). However as the study validity criteria were met and no significant effects were observed in any case, the RMS does not consider this to be a major deviation from guidance.

The agreed endpoint considered suitable for use in the risk assessment is:

- **3 h EC_{50} = >1000 mg a.s./L**

B.9.9. MONITORING DATA

None submitted or required.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

See Volume 3 (PPP) section B.9.4.2 (Exposure via groundwater), where the risk from metabolites occurring in ground water is considered.

B.9.11. LITERATURE REVIEW

B.9.11.1 Literature review of BAS 750 F and related compounds

A literature search was performed on the parent molecule and the discovered metabolites from the soil and aquatic compartments. But, being a new active substance, no references in the literature were found for BAS 750 F. Literature search was also extended to metabolites and checked for relevance. A full set of the found references and assessment for relevance may be found in an Excel file attached to M-CA Section 9.

The search process is documented in all details with search profiles, search histories and summary tables. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092.

Evaluation of studies

The process of selection of relevant scientific peer-reviewed open literature was done in two steps:

The First Selection step for relevance based on summary records (e.g. titles, abstracts, index terms, keywords) was done by the Agro Information Professionals.

- Obviously irrelevant records were tagged as “Ballast”. This ballast was controlled by scientific experts in the corresponding subject areas but was not further processed.
- Summary records which appear to be relevant and those of unclear relevance were tagged as “Hit” and went to the next level of evaluation.

The Second Detailed Assessment was done by the scientific experts in the corresponding areas.
Records tagged as “Hit” were further evaluated in depth. To facilitate a comprehensible listing of the "Hits" in Ecotoxicology and the other sections, an Excel file was generated for each section with 3 typical registers, namely:

- "no relevant endpoint"
- "evaluated - not-relevant"
- "used for dossier"

In a first step (rapid assessment) the "Hits" were reviewed based on the information given in the title and the abstract with regard to relevance for the regulatory endpoints in the respective regulatory area. Those records which were clearly judged as not assignable to any regulatory endpoint were shifted into the register "no relevant endpoint" with an explaining reasoning.

In a second step (detailed assessment), all remaining records were assessed in detail based on the complete report by the respective expert(s) and separated into relevant reports for further discussion and those clearly not relevant.

Databases Searched

Only three databases have been searched (CAPLUS, BIOSIS and CABA) potentially limiting the range of studies that could be located. The reason why each database was selected is presented in Table B.9.11-1. There was no time limitation on the date span searched for the review, and covered 1907, 1926 and 1973 to the day of the search for CAPLUS, BIOSIS and CABA respectively. This covers the minimum requirement of 10 years prior to the date of the search.

Table B.9.11.1-1 Databases searched as p[art of the BAS 750 F literature review

Database:	**Total**	**CAPLUS Chemical Abstracts Plus**	**BIOSIS**	**CAB Abstracts**
Provider:		STN International	STN International	STN International
Justification for choosing the source: **- for STN databases referring to STN database summary sheets**		The Chemical Abstracts (CA) database covers all areas of Biochemistry, Chemistry and Chemical engineering, and related sciences. Sources include over 8,000 journals, patents from 38 national patent offices and two international patent organizations, technical reports, books, conference proceedings, and dissertations. Electronic only journals and Web preprints are also covered. Bibliographic terms, indexing terms, roles, CAS Registry Numbers, International Patent Classification, and abstracts are searchable.	BIOSIS Previews® is the largest and most comprehensive life science database in the world. Amongst others subject coverage includes Agriculture, Biochemistry, Biophysics, Botany, Environmental Biology, Physiology, Toxicology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are all searchable.	The CAB Abstracts database covers worldwide literature from all areas of agriculture and related sciences including Agriculture, Agricultural chemicals, Animal sciences and production, Crop protection, Crop sciences and production, Environment, Soils and fertilizers. Sources for CABA include journals, books, reports, published theses, conference proceedings, and patents. Bibliographic information, indexing terms, abstracts, and CAS Registry Numbers are searchable.
Date span of the source:		1907 – to present	1926 – to present	1973 – to present
Date of main search:		2015-12-04	2015-12-04	2015-12-04
Date span of the search:		no time limitation	no time limitation	no time limitation
Date of the latest database update included in the search:		20151203/UP	20151202/UP	20151202/UP

Search parameters

The CAS numbers searched covered the active, relevant metabolites and mixtures containing the active substance. Additionally, common and trade names for the active were searched for. The RMS notes that searching for the common names of relevant metabolites may broaden the results.

The chemical terms were then partnered with search terms of relevance to ecotoxicology and the environment. In general the terms include a variety of family names and common test species, in addition to some more generic terms such as insect, mesocosm, and bird. However order level searches were not included for invertebrates. The aquatic search included a range of test species, while also allowing for more general species and additionally searches for amphibians.

For non-avian vertebrate species, family level mammal terms are limited to the Rodentia and Lagomorpha and the only species term not covered by either of these two families is the shrew. Other than these issues, the additional search terms appear to cover a comprehensive range relating to those taxa considered as part of the ecotoxicological risk assessment. In addition, relevant guidelines were included within the search.

The search terms used are presented in Table B.9.11-2

Table B.9.11-2 List of search terms used for the ecotoxicology section of the report

Resulting CAS Registry numbers for search:
BAS 750 F including isomers:
(1417782-03-6)
1616236-94-2 or 1652606-36-4

BAS 750 F metabolites:
(330-60-9 or 114419-45-3 or 333354-47-5 or 4819-36-7 or 28711-29-7 or 1450828-63-3 or 64882-52-6 or 17681-00-4 or 3233-55-4 or 653605-30-2 or 120161-35-5 or 55064-81-8 or 3233-66-7)

(1H)1,2,4-Triazole was searched as an additional metabolite:
(288-88-0)

BAS 750 F mixtures:
(1616237-33-2 or 1616237-34-3 or 1616237-35-4 or 1616237-36-5 or 1616237-37-6 or 1616237-38-7 or 1616237-39-8 or 1616237-40-1 or 1616237-41-2 or 1616237-42-3 or 1616237-43-4 or 1616237-44-5 or 1616237-45-6 or 1616237-46-7 or 1616237-47-8)
(1616237-48-9 or 1616237-49-0 or 1616237-50-3 or 1616237-51-4 or 1616237-52-5 or 1616237-53-6 or 1616237-54-7 or 1616237-55-8 or 1616237-56-9 or 1616237-57-0 or 1616237-58-1 or 1616237-59-2 or 1616237-60-5 or 1616237-61-6 or 1616237-62-7)
(1616237-63-8 or 1616237-64-9 or 1616237-65-0 or 1616237-66-1 or 1616237-67-2 or 1616237-68-3 or 1616237-69-4 or 1616237-70-7 or 1616237-71-8 or 1616237-72-9 or 1616237-73-0 or 1616237-74-1 or 1616237-75-2 or 1616237-76-3 or 1616237-77-4)
(1616237-78-5 or 1616237-79-6 or 1616237-80-9 or 1616237-81-0 or 1616237-82-1 or 1616237-83-2 or 1616237-84-3 or 1642544-31-7 or 1703753-82-5 or 1805789-50-7 or 1809504-09-3 or 1809504-10-6 or 1809504-11-7 or 1809504-12-8 or 1809504-13-9)
(1809504-14-0 or 1809504-15-1 or 1809504-16-2 or 1809504-17-3 or 1809504-18-4 or 1809504-19-5 or 1809504-20-8 or 1809504-21-9 or 1809504-22-0 or 1809504-23-1 or 1809504-24-2 or 1809504-25-3 or 1809504-26-4 or 1809504-27-5 or 1809504-28-6)
(1809504-29-7 or 1809504-30-0 or 1809504-31-1 or 1809504-32-2 or 1809504-33-3 or 1809504-34-4 or 1809504-35-5 or 1809504-36-6 or 1809504-37-7 or 1809504-38-8 or 1809504-39-9 or 1809504-40-2 or 1809504-41-3 or 1809504-42-4 or 1809504-43-5)
(1809504-44-6 or 1809504-45-7 or 1809504-46-8 or 1809504-47-9 or 1809504-48-0 or 1809504-49-1 or 1809504-50-4 or 1809504-51-5 or 1809504-52-6 or 1809504-53-7 or 1809504-54-8 or 1809504-55-9 or 1809504-56-0 or 1809504-57-1 or 1809504-58-2)
(1809504-59-3 or 1809504-60-6 or 1809504-61-7 or 1809504-62-8 or 1809504-63-9 or 1809504-64-0 or 1809504-65-1 or 1809504-66-2 or 1809504-67-3 or 1809504-68-4 or 1809504-69-5 or 1809504-70-8 or 1809504-71-9 or 1809504-72-0 or 1809504-73-1)
(1809504-74-2 or 1809504-75-3 or 1809504-76-4 or 1809504-77-5 or 1809504-78-6 or 1809504-79-7 or 1809504-80-0 or 1809504-81-1 or 1809504-82-2 or 1809504-83-3 or 1809504-84-4 or 1809506-63-5 or 1809506-64-6 or 1809506-65-7 or 1809506-66-8)
(1809506-67-9 or 1809506-68-0 or 1809506-69-1 or 1809506-70-4 or 1809506-71-5 or 1809506-72-6 or 1809506-73-7 or 1809506-74-8 or 1809506-75-9)

Search of common and trade names:

BAS750F or BAS750 or BAS(w)(750F or 750)

Search strategy for data:

in CAPLUS, CABA, BIOSIS:

Substances AND

****Ecotox general****

- (bioavail? or biotransform? or biodegrad? or bioaccumul? or bio(w)accumul? or BAF or bioconcentrat? or bio(w)concentrat? or BCF or biomagnif? or bio(w)magnif? or BMF or biomonit? or food(1a)chain# or dietary(1a)exposur?)
- ((bio# or biolog?)(w)(avail? or transform? or degrad? or accumulat? or concentrat? or concn# or magnif? or monitor?))
- (ecotox? or ecolog? or ecosystem? or biosph?)
- ((eco or bio?)(w)tox? or biotoxic?)
- (side(w)effect#)

- (fauna# or microfauna# or microflora# or macrofauna# or macroflora# or mesofauna# or mesoflora# or (micro or macro or meso)(w)(fauna# or flora#) or bacteria# or (macro or micro)(w)organism# or macroorganism# or microorganism#)
- (beneficial# or non(w)target or nontarget or predator# or predac!ous or natural(w)enem###)
- (NOEC or NOEL or NOER or EC_{50} or ER50 or LD_{50} or LC_{50} or NOAEC or NOEAEC)
- (species(w)sensitiv?(w)distribution# or SSD or SSDs)
- (toxic?(w)(endpoint# or threshold#))
- ((lab?)(3a)(study or studies))
- ((lab or laboratory or field)(w)(condition# or test? or bioassay# or method# or assessment#))
- (test?(1a)chemical# or (Organization(1w)Economic(w)Cooperation(1w)Development or OECD or Office(1w)Prevention(w)Pesticides(1w)Toxic(w)Substances or OPPTS)(5a)(test? or guideline#))

Specific results of Ecotox Wildlife, Terrestric and Aquatic were subtracted from results of Ecotox general

****Ecotox Wildlife****

- (wildlife#)

- (bird# or AVES or avian)

- (duck# or mallard# or anas)
- (chicken# or gallus or chick# or pullet# or hen#)

- (blackbird# or merle# or ouzel# or Turdus and merula)
- (thrush## or Turdidae or Turdus)
- (Blackcap# or Sylvia and atricapilla or Sylvia or sylviid(w)warbler#)
- (Black(w)Redstart# or Phoenicurus and ochruros or Phoenicurus)
- (Blue(w)Tit# or Bluetit# or nun# or tomtit# or (Cyanistes or Parus) and caeruleus or Parus or Paridae or Parus)
- (Chaffinch## or Fringilla and coelebs or Fringilla)
- (dunnock# or prunella and modularis or Prunella or Hedge(w)(Sparrow# or Accentor# or Warbler#))
- (goldfinch## or gold(w)finch## or Carduelis and carduelis or Carduelis or Carduelinae)
- (linnet# or (Acanthis or Carduelis) and cannabina or Carduelis or Carduelinae)
- (Partridge# or pheasant# or Phasianus or Perdix)
- (Phasianidae or Phasianinae or Odontophorinae or Perdicinae)
- (Pratincole# or Greywader# or grey(w)wader# or Glareola or Stiltia or Glareolidae)
- (quail# or coturnix)

- (Serin or Serins or Serinus)
- (wagtail# or wag(w)tail# or Motacilla and alba or Motacillidae or Motacilla)
- (warbler# or phylloscopus or Phylloscopidae)
- (woodlark# or Lullula and arborea or lullula)
- (Zebra(w)finch## or Taeniopygia and guttata or Taeniopygia or Poephila or estrildid(w)finch## or estrildidae or estrilididae)

- (OECD(5a)205 or OECD205 or OECD(5a)206 or OECD206 or OECD(5a)416 or OECD416 or OPPTS(5a)850.2100 or OPPTS850.2100 or OPPTS(5a)850.2200 or OPPTS850.2200 or OPPTS(5a)850.2300 or OPPTS850.2300)

- (rat or rats or rattus)
- (rodent# or rodentia)
- (muridae or murinae or mouse or mice or mus)
- (rabbit# or hare# or lagomorph# or leporidae or lepus)
- (vole# or microtus)
- (shrew# or sorex or soricidae)

****Ecotox Terrestric (below ground)****

- (invertebrat? or earthworm# or earth(w)worm# or lumbric? or eisenia)
- (soil#(5a)(organism# or microorganism# or arthropod# or mite# or fung## or function# or respiration#) or nitrogen##(w)transform?)
- (Collembola# or springtail# or spring(w)tail# or Folsomia or Entomobryidae or Isotomidae or Mesostigmata or Cryptostigmata or Hypoaspis)

- (OECD(5a)207 or OECD207 or OECD(5a)216 or OECD216 or OECD(5a)217 OR OECD217 or OECD(5a)222 or OECD222 or OECD(5a)226 or OECD226 or OECD(5a)232 or OECD232 or ISO(5a)11268-3 or ISO11268-3 or ISO(5a)11267? or ISO11267? or ISO(5a)17512? or ISO17512?)

****Ecotox Terrestric (above ground)****

- (Arachnid? or mite# or acari###)
- ((web or wolf)(w)spider# or Lycosidae or Pardosa or Allopecosa or Lycosa or Pirata or Oribatidae or Phytoseiidae or Typhlodromus or Amblyseius or Phytoseius)

- (insect# or bee or bees or honeybee# or apis or pollinator# or Bumblebee# or bumble(w)bee# or bombus or osmia or megachile or megachilidae)
- (Vanessa or Parnassius or Aglais or Inachis or Papillio)
- (Pieris or Lobesia or Ostrinia or Trichoplusia or Heliothis or Spodoptera or Mamestra)

- (Parasitoid# or parasitic(w)wasp# or Aphidius or Aphidiinae or Braconidae or Aphelinidae or Aphelinus or Encarsia or Trichogrammatidae or Trichogramma)
- (Chrysoperla or Chrysopidae or Chrysopa or Hermerobiidae)
- (Anthocoridae or Anthocoris or Orius)
- (Coccinellidae or Coccinella or Adalia or Harmonia or Calvia or Propylea)
- (Carabidae or Poecilus or Carabus or Calosoma or Amara or Harpalus or Pterostichus or Abax)
- (Staphylinidae or Aleochara and bilineata)

- (ESCORT(w)2 or ESCORT2 or ESCORT(w)II or ESCORTII)

****Ecotox Terrestric (above ground - Standard Test Vascular Plants)****

- ((terrestri? or non(w)target or nontarget)(w)plant#)
  ((allium or porrum or vulgaris) and cepa or onion#)
  (avena and sativa or oat#)
  (beta and vulgaris or beet# or sugarbeet# or fodderbeet# or mangel# or redbeet# or beetroot# or redroot# or red(a)root#)
  (brassica and (napus or oleifera) or rape# or rapeseed# or colza# or canola#)
  (brassica and oleracea or cabbage# or kale# or cauliflower# or broccoli# or calabrese# or brussel#(a)sprout# or toy or kohlrabi# or (kale or stem or hungarian or rooted or cabbage)(a)turnip# or pakchoi# or pak(w)choi#)
  (cucumis and (sativus or esculentus) or cucumber)
  (daucus and carota and sativus or carrot#)
  (glycine and (max or hispida or soja) or phaseolus and max or ((dolichos or hispida) and soja) or sojbean# or sojabean# or soy or soya or soybean# or soyabean#)
  (lactuca and sativa or lettuce# or salad# or Iceberg lettuce# or oak leaf lettuce# or lollo rosso# or Batavian#)
  (linum and usitatissimum or flax##(a)(common or cultivated or linen) or linseed# or (lin or linnen)(a)seed#)
  (lycopersic## and (esculentum or lycopersicum) or solanum and lycopersicum or tomato## or love(w)apple#)
  (Lolium and perrenne or ryegrass## or rye(w)grass##)
  (pisum and sativum or pea or peas)
  (zea and (mays or vulgaris) or maize or corn)

  ---AND ---

  (Seedling(3a)emerg? or vegetative(w)vigo!r# or vigo!r# or plant(w)weight# or biomass or plant(w)survival# or phytotox? or phyto(w)tox? or NOEC or ER50 or Non(w)target(w)(plant# or weed# or crop#) or adjacent(w)crop#)

- (OPPTS(5a)(850.4100 or 850.4150 or 850.4200 or 850.4225 or 850.4230 or 850.4250 or 850.4300) or OPPTS850.4100 or OPPTS850.4150 or OPPTS850.4200 or OPPTS850.4225 or OPPTS850.4230 or OPPTS850.4250 or OPPTS850.4300)
- (OECD(5a)(208 or 227) or OECD208 or OECD227)

****Ecotox Aquatic****

- (mesocosm## or microcosm## or macrocosm## or (meso or micro or macro)(w)cosm##)
- (ELINK or HARAP or aquatic(w)(exposure# or effect# or risk(w)assessment#))
- (hazard(w)concentration# or HC1 or HC(w)1 or HC_5 or HC(w)5 or lower(w)limit#)

- ((freshwater# or water# or aquatic or sediment#)(3a)(organism# or animal# or plant# or invertebrate# or macroinvertebrate# or biota# or arthropod# or insect# or snail#) or aquatic(w)environment? or pelagic or benth##)

- (Chironomid# or chironomidae or Chironomus and riparius or Chaoborus)
- (Crustace## or phyllopoda# or cladocer? or ?daphn? or waterflea?)
- (Mysid(w)shrimp# or Mysidopsis and bahia or ?mysid?)

- (Tubifex or benth?(1a)(oligochaet? or macrofauna# or macro(w)fauna#))

- (Oyster# or Crassostrea and virginica)

- Procambarus

- (fish## or PISCES)
- (pimephales or minnow#)
- (cyprinodon and variegatus or (sheepshead or sheeps(w)head)(a)minnow#)
- (cyprinus and carpio or carp#)
- (Oncorhynchus and mykiss or rainbow(a)trout# or trout# or salmo and (gardneri or irideus))
- (lepomis and (auritus or macrochirus or gibbosus or cyanellus) or (orangespotted or readbreast or yellowbelly or green or pumpkinseed)(w)sunfish## or bluegill#)
- (brachydanio and rerio or zebrafish## or danio or rerio)

- (Amphibia# or tadpole# or xenopus or frog# or toad#)

- (reptile# or reptilia or reptiliae)

- (?plankton? or ?alga or ?algae or chlorophyt? or Selenastrum or Pseudokirchneriella or Scenedesmus or Ankistrodesmus or Desmodesmus or Chlorella)

- (Dinophyt? or flagellate#)
- (bacillariophyc? or diatom? or Navicula)
- (cyanobacteri? or Anabaena)
- (Eugleno? or Euglena)
- (Skeletonema or Periphyton)
- (Cryptophy? or Chroomonas or cryptomonas or Ankyra)

- (macrophyt? or macro(w)phyt? or lemna## or lemnaceae or Potamogeton or pondweed# or pond(w)weed# or Chara or Ceratophyllum or hornwort# or horn(w)wort# or elodea or waterweed# or water(w)weed#)
- (submer? or emergent?)
- (Water(w)milfoil# or Myriophyllum)

- (Alga# or OECD(5a)201 or OECD201 or OPPTS(5a)850.5400 or OPPTS850.5400)
- (Amphibian(w)metamorphosis(w)assay or Xenopus or OECD(5a)231 or OECD231 or OPPTS(5a)890.1100 or OPPTS850.1100)
- (Chironomus(w)acute(w)spiked(w)sediment or OECD(5a)218 or OECD218)
- (Chironomus(w)acute(w)spiked(w)water or OECD(5a)219 or OECD219 or OPPTS(5a)850.1790 or OPPTS850.1790)
- (Chironomus(w)chronic or OECD(5a)233 or OECD233)
- (Chironomus(w)acute or OECD(5a)235 or OECD235)

- (Daphni#(w)acute or OECD(5a)202 or OECD202 or OPPTS(5a)850.1010 or OPPTS850.1010)
- (Daphni#(w)chronic or OECD(5a)211 or OECD211 or OPPTS(5a)850.1300 or OPPTS850.1300)
- (Fish(w)acute or OECD(5a)203 or OECD203 or OPPTS(5a)850.1075 or OPPTS850.1075)
- (Fish(w)assay(w)21(w)d or OECD(5a)230 or OECD230)
- (Fish(w)BCF or OECD(5a)305 or OECD305 or OPPTS(5a)850.1730 or OPPTS850.1730)
- (Fish(w)ELS or OECD(5a)210 or OECD210 or OPPTS(5a)850.1400 or OPPTS850.1400)
- (Fish(w)FLC or OPPTS(5a)850.1500 or OPPTS850.1500)
- (Fish(w)juvenile(w)28(w)d or OECD(5a)215 or OECD215)
- (Fish(w)prolonged(w)14(w)d or OECD(5a)204 or OECD204)
- (Fish(w)short(w)term(w)embryo or OECD(5a)212 or OECD212)

- (Fish(w)short(w)term(w)reproduction(w)assay or OECD(5a)229 or OECD229)
- (Lemna or OECD(5a)221 OECD221 or OPPTS(5a)850.4400 or OPPTS850.4400)
- (Microcosm(w)generic or OPPTS(5a)850.1900 or OPPTS850.1900)
- (Microcosm(w)site(w)specific or OPPTS(5a)850.1925 or OPPTS850.1925)
- (Mysid(w)acute or OPPTS(5a)850.1035 or OPPTS850.1035)
- (Mysid(w)chronic or OPPTS(5a)850.1350 or OPPTS850.1350)
- (Oyster(w)acute or OPPTS(5a)850.1025 or OPPTS850.1025)

Relevance and reliability

The relevance of literature studies has been defined as the extent to which a test is appropriate for a particular hazard or risk assessment, the way a study can be used and the framework used for evaluation hence a study may be relevant in one framework but not in another. The following criteria have been used in order to evaluate the relevance of the literature data:

- **Selection**: Do not set criteria too narrow.
- **Evaluate**: Title, Abstract, and Full article, if needed.
- **Test material**: The test material is the primary criterion to consider (EFSA 2011, **Appendix A**; AGES 2013, **Appendix B**), because it determines the framework of each application for registration. Applying pure logic, only when the test material is concordant or identical to the one under evaluation, a peer-reviewed article should be further evaluated. However, this is a weak point of many published schemes is that they are inconclusive on the hierarchy of the proposed categories and criteria (e.g. Kase et al. 2012, Kase (2015).
- **Data requirements**: Consider Areas (**see section 5.2.5**) and related requirements being relevant for ecotoxicology (see **Attachment D**).
- **Endpoint / Descriptive result**: High / low probability of being relevant.
- **Field studies**: European conditions (climate, species, …) are relevant.
- **Secondary literature** (e.g. review, books, etc.): To be excluded.
- **Confirmatory data** (studies without new relevant data): To be excluded.
- **Non-European species** should not be excluded.
- **Additional criteria**: To be handled flexible - Meet the needs of the active substance.

The reliability of the study has been defined as the inherent quality of a study, thus the criteria will always be the same in whatever framework reliability is evaluated, the reporting quality of methodology, experimental procedure, and results (i.e. free from bias, findings reflect true facts), and the reproducibility of the study. According to these, the reliability of the study was concluded.

Results

The results of the search method employed by the applicant is presented in Table B.9.11.1-1. CABA was notable for finding no hits at all, raising question on how useful the database, and considering that only three databases have been searched, the impact of an irrelevant database is greater.

Table B.9.11.1-2 Summary of ecotoxicological literature review search results

Database	Total	CAPLUS	BIOSIS	CABA
Total number of summary records for BAS 750 F and Metabolites / (1H)1,2,4-Triazol Metabolite retrieved:	**164**	20 / 119	1 / 24	0 / 0
Total number of summary records after removing duplicates:	**162 (20/142)**	20 / 119	0 / 23	0 / 0
Total number of summary records retrieved after first selection step:	**15 (1 / 14)**	1 / 13	0 / 1	0 / 0
Category: Ecotox	**15**			
Category: Ecotox Ballast	**147**			

Table B.9.11.1-3: Summary of the 15 potentially relevant literature studies identified

Title	Abstract	Conclusion
The metabolism of phenol and substituted phenols in zebra fish	The metab. of 5 phenols in zebra fish (Brachydanio rerio) was studied after uptake from the medium. The results showed no qual. differences to other Cyprinid fish species, only the oxidn. rate seemed to be lower. Ph glucuronide, Ph sulfate, and quinol sulfate were identified as metabolites of phenol. Identified metabolites of 2-cresol (I) were 2-cresyl glucuronide, 2-cresyl sulfate, and 2-hydroxybenzoic acid in trace amts. Only the glucuronide and sulfate conjugates were detected as metabolites of 4-nitrophenol, 4-chlorophenol, and pentachlorophenol.	Rejected. The test substance was not relevant and did not require a risk assessment
The effect of nitrification inhibitors on nitrous oxide emissions from cattle urine depositions to grassland under summer conditions in the UK	Nitrous oxide (N2O) has become the prime ozone depleting atm. emission and the third most important anthropogenic greenhouse gas, with a global warming potential approx. 300 times higher than CO2. Nitrification and denitrification are processes responsible for N2O emission from the soil after nitrogen input. The application of a nitrification inhibitor can reduce N2O emissions from these processes. The objective of this study was to assess the effect of two different nitrification inhibitors (dicyandiamide (DCD) and a com. formulation contg. two pyrazole derivs. (PD), 1H-1,2,4-triazole and 3-methylpyrazole) on N2O emissions from cattle urine applications for summer grazing conditions in the UK. Expts. were conducted under controlled conditions in a lab. incubator and under field conditions on a grassland soil. The N2O emissions showed similar temporal dynamics in both expts. DCD concn. in the soil showed an exponential degrdn. during the expt., with a half-life of the order of only 10 d (air temp. c. 15 .degree.C). DCD (10 kg ha-1) and PD at the highest application rate (3.76 kg ha-1) reduced N2O emissions by 13% and 29% in the incubation expt. and by 33% and 6% in the field expt., resp., although these redns. were not statistically significant ($P > 0.05$). Under UK summer grazing conditions, these nitrification inhibitors appear to be less effective at reducing N2O emissions than reported for other conditions elsewhere in the literature, presumably due to the higher soil temp.	Rejected. The test substance was not relevant and did not require a risk assessment
Triazole-induced toxicity in developing rare minnow (Gobiocypris rarus) embryos	Using rare minnow (Gobiocypris rarus) at early-life stages as exptl. models, the developmental toxicity of five widely used triazole fungicides (myclobutanil, fluconazole, flusilazole, triflumizole, and epoxiconazole) were investigated following exposure to 1-15 mg/L for 72 h. Meanwhile, morphol. parameters (body length, body wt., and heart rate), enzyme activities (superoxide dismutase (SOD), glutathione S-transferase (GST), ATPase (ATPase), and acetyl cholinesterase (AChE)), and mRNA levels (hsp70, mstn, mt, apaf1, vezf1, and cyp1a) were also recorded following exposure to 0.2, 1.0, and 5.0 mg/L for 72 h. Results indicated that increased malformation and mortality, decreased body length, body wt., and heart rate provide a concn.-dependent pattern; values of 72 h LC_{50} (median lethal concn.) and EC_{50} (median effective concn.) ranged from 3 to 12 mg/L. Most importantly, the results of the present study suggest that even at the lowest concn., 0.2 mg/L, five triazole fungicides also caused notable changes in enzyme activities and mRNA levels. Overall, the present study points out that those five triazole fungicides are highly toxic to the early development of G. rarus embryos. The information presented in this study will be helpful in better understanding the toxicity induced by triazole fungicides in fish embryos.	Rejected. The test substance was not relevant and did not require a risk assessment
Effects of current-	Fungicides are frequently applied in agriculture and are subsequently detected in	Rejected. The

use fungicides and their mixtures on the feeding and survival of the key shredder Gammarus fossarum	surface waters in total concns. of up to several tens of micrograms per L. These concns. imply potential effects on aquatic communities and fundamental ecosystem functions such as leaf litter breakdown. In this context, the present study investigates sublethal and lethal effects of org. (azoxystrobin, carbendazim, cyprodinil, quinoxyfen, and tebuconazole) and inorg. (three copper (Cu)-based substances and sulfur) current-use fungicides and their mixts. on the key leaf-shredding invertebrate Gammarus fossarum. The feeding activity of fungicide-exposed gammarids was quantified as sublethal endpoint using a static (org. fungicides; 7 d test duration) or a semi-static (inorg. fungicides; 6 d test duration with a water exchange after 3 d) approach (n = 30). EC_{50}-values of org. fungicides were generally obsd. at concns. resulting in less than 20% mortality, with the exception of carbendazim. With regard to feeding, quinoxyfen was the most toxic org. fungicide, followed by cyprodinil, carbendazim, azoxystrobin, and tebuconazole. Although all tested org. fungicides have dissimilar (intended) modes of action, a mixt. expt. revealed a synergistic effect on gammarids' feeding at high concns. when using 'independent action' as the ref. model (.apprx.35% deviation between predicted and obsd. effect). This may be explained by the presence of a synergizing azole fungicide (i.e. tebuconazole) in this mixt. Furthermore, lethal concns. of all Cu-based fungicides assessed in this study were comparable amongst one another. However, they differed markedly in their effective concns. when using feeding activity as the endpoint, with Cu-sulfate being most toxic, followed by Cu-hydroxide and Cu-octanoate. In contrast, sulfur neither affected survival nor the feeding activity of gammarids (up to .apprx.5 mg/L) but reduced Cu-sulfate's toxicity when applied in a binary mixt. Sulfur-related metabolic processes which reduce the physiol. availability of Cu may explain this antagonistic effect. For both fungicide mixts., the present study thus uncovered deviations from the appropriate ref. model, while ecotoxicol. effects were obsd. at field relevant (total) fungicide concns. Addnl., for more than half of the tested single substances, a potential risk for Gammarus and thus for the ecol. function mediated by these organisms was evident at concns. measured in agriculturally influenced surface waters. These results suggest that risks to the fundamental ecosystem function of leaf litter breakdown posed by fungicides may not be adequately considered during the regulation of these compds., which makes further exptl. efforts necessary.	test substance was not relevant and did not require a risk assessment
The Enantioselective Pharmacokinetics Metabolism of Diniconazole in Quail (Coturnix coturnixs japonica)	The pharmacokinetics of diniconazole enantiomers in quail (Coturnix coturnix japonica) were investigated by liq. chromatog.-tandem mass spectrometry (LC-MS/MS). Quails were exposed to racemic diniconazole in capsule by oral at dose of 10 mg/kg (body wt.). The maximal concns. obsd. in blood, heart, liver, and kidney were 3.18, 11.35, 12.32, 15.03 mu.g/g for S-diniconazole, and 1.13, 3.70, 6.00, 2.60 .mu.g/g for R-diniconazole. The elimination of enantiomers all met the one-compartment model in blood, heart, liver, and kidney well. The elimination half-lives (T1/2) of S-diniconazole were 2.87, 3.85, 5.29, and 4.42 h in blood, heart, liver, and kidney, resp.; the T1/2 of R-diniconazole were 2.44, 3.42, 146.23, and 74.02 h in blood, heart, liver, and kidney, resp. The enantiomer fractions (EFs) steadily increased from 0.50 to 0.92 in blood samples and 0.91 in heart samples. Meanwhile, the values increased to 0.70 and 0.80 in liver and kidney initially, and then decreased to 0.33 and 0.44 at the end of the expt. Metab. was examd. as well and it was found that diniconazole was metabolized to 1, 2, 4-triazole, (E)-3-(1H-1, 2, 4-triazol-1-yl) acrylaldehyde, (E, S)-(R, S)-4-(2, 4-dichlorophenyl)-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-ene-1, 3-diol, (E)-4-(2, 4-dichlorophenyl)-3-hydroxy-2, 2-dimethyl-5-(1H-1, 2, 4-triazol-1-yl) pent-4-enoic acid, and 1, 3-dichlorobenzen in all samples of quail. Chirality 00:000-000, 2013. .COPYRGT. 2013 Wiley Periodicals, Inc.	Rejected. The test substance was not relevant and did not require a risk assessment
Conclusion on the peer review of the pesticide risk assessment of the active substance ipconazole	A review. The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State the United Kingdom, for the pesticide active substance ipconazole are reported. The context of the peer review was that required by Commission Regulation (EU) No. 188/2011. The conclusions were reached on the basis of the evaluation of the representative uses of ipconazole as a fungicide for seed treatment of wheat and barley. The reliable endpoints concluded as being appropriate for use in regulatory risk assessment, derived from the available studies and literature in the dossier peer reviewed, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.	Rejected. Review article on a test substance was that did not require a risk assessment
Conclusion on the peer review of the pesticide risk	A review. The EFSA has been requested to deliver its conclusions on fenbuconazole. The conclusions are reached on the basis of the evaluation of the representative uses of fenbuconazole as a fungicide on wheat, apples and grapes.	Rejected. Review article.

assessment of the active substance fenbuconazole	No areas of concern are identified in the mammalian toxicol. and residue sections. Regarding the environmental fate and behavior of fenbuconazole, no specific data gaps other than addressing potential preferential enantio-selective degrdn. are identified. No areas of concern are identified with respect to the potential for groundwater contamination. A high risk is identified for aquatic organisms, and risk mitigation measures such as no-spray buffer zones are required.	
2D-QSAR study of the acute toxicity effects of triazole pesticides on D. magna	The acute immobilization toxicity effects expressed as pI50 (-lg EC_{50}) of sixteen triazole pesticides on D. magna in 48 h were studied. 2D-QSAR models were carried out with some quant. chem. parameters calcd. by GO3W program with 6-31G basis, including the energy of the HOMO (EHOMO) and energies of its two neighborhood orbits (EHOMO-1 and (EHOMO-2), the energy of the lowest unoccupied mol. orbit(ELUMO) and energies of its two neighborhood orbits (ELUMO+1 and ELUMO+2), at. charges, electrostatic potencial charges, dipole moment, the first-order polarizability, the first-order hyperpolarizability, lipid/water partition coeff., heat of formation, hydration energy, molar refractivity etc. These models would provide the theor. basis for predicting the pI50 of the compds. with a similar structure. Addnl., the predicting models could initially explain the mechanism of toxicity to D. magna, and scientifically design triazole pesticides with high efficiency and low toxicity from perspective of mol. structure.	Rejected. The QSAR data within the study is superseded by GLP studies or QSAR data submitted by BASF
Acute toxicity test of agricultural pesticides on silver catfish (Rhamdia quelen) fingerlings	Toxicity risks of agricultural pesticides to fishes are pivotal. Currently, many questions remain unsolved regarding the toxicity of commonly used pesticides to silver catfish (R. quelen), a South American catfish. The present studies have been designed to investigate the acute toxicity and the lethal concn. (LC_{50}) of 4 herbicides, 2 fungicides, and 2 insecticides to silver catfish fingerlings. All expts. were carried out in triplicates, in a static bioassay system, using com. available pesticides. The data was analyzed through the Trimmed Spearman-Karber method available from the Environmental Protection Agency. The 96-h LC_{50} and 95% lower and upper confidence limits, resp., for the following pesticides were detd.: glyphosate (7.3 mg L-1; 6.5-8.3), atrazine (10.2 mg L-1; 9.1-11.5), atrazine + simazine (10.5 mg L-1; 8.9-12.4), mesotrione (532.0 mg L-1; 476.5-594), tebuconazole (5.3 mg L-1; 4.9-5.7), methyl parathion (4.8 mg L-1; 4.3-5.3), strobulurin, and triazole (9.9 mg L-1; 8.7-11.2). Diflubenzuron was also tested and caused no fish mortality up to 1 g L-1. The toxic concn. of these pesticides to silver catfish fingerlings fell above the concn. used for application in the field and, except following accidental application or misplacing of empty recipients, it should not cause fish mortality. Nonetheless, the data obtained will be useful to study the long-term effect of these products on the hematol., biochem., hormonal, and immunol. parameters of silver catfish and related fish species in South Brazil.	Rejected. The test substance was not relevant and did not require a risk assessment
Some biochemical parameters of blood plasma of turkey-hens following administration of 1,2,4-triasole derivative	The present study involved 180 slaughter turkey-hens of heavy Big-6 type divided into four groups (in triplicate repetition for 15 birds). All the birds were fed with the same std. full-dose mixts. in 5-stage system. The turkey-hens of groups I, II and III were given 1,2,4-triasole deriv. (3-(2-pyridil)-4-phenyl-1,2,4-triasole-5-carboxylic acid), which has antibacterial, antifungal and immunomodulating properties, in amt. of 50, 75 and 100 .mu.g per 1 dm3 of water. Group IV - control was given water without the additive. The 1,2,4-triasole deriv. was given to drinking water, starting from the first day of bird's life and for the whole rearing period. The present results of biochem. anal. of blood plasma showed that addn. of examd. substance significantly reduced concn. of protein, glucose, triglycerides and uric acid as compared to control. It was stated that tested 1,2,4-triasole deriv. elevated the level of HDL fraction percentage and alk. phosphatase activity in blood plasma.	Rejected. The endpoint from the study, blood parameters in birds, is of no relevance to setting regulatory endpoints
The influence of anti-corrosion compounds on algal growth	At present, ecological considerations have more impact on the inhibitor choice than inhibition efficiency itself. In the presented study the biological toxicity assays on the green alga Scenedesmus quadricauda (Turp.) Bréb. Strain Greifswald/15 were performed. Toxicity of six corrosion inhibitors was tested. The toxicity of the tested chemicals is increasing in the order: dibenzylsulfoxide < propargylalcohol < benzimidazole < 1,2,3-benztriazole < propargylbenzoate < 1,2,4-triazole. Moreover carcinogenic and teratogenic impacts of the inhibitors were investigated. More detailed ecotoxicological tests should be performed as cancerous and morphological (mutagenic) impacts of some of the studied compounds on alga cells were found in the presented study.	Not rejected. Considered further in Table B.9.11.2-5 below.
Results of the development of a new nitrification inhibitor for	Combination activity was evaluated toxicol. and ecotoxicol. of the nitrification inhibitor dicyandiamide (DCD) + 1H-1,2,4-triazole (TZ). Application of DCD +TZ (10:1) in combination with carbamide- resp. NH4-contg. N fertilizers resulted in a decreased NO3 output into the soil (35-48%), decrease of N2O emission (<90%),	Rejected. The test substance was a combination

ammonium stabilization	and increase of yield in agriculture. The nitrification inhibitor DCD + TZ (10:1) was suitable for utilization with org. fertilizer (i.e. manure).	
The acute oral toxicity, repellency, and hazard potential of 998 chemicals to one or more species of wild and domestic birds	The acute oral toxicity, repellency, and hazard potential of 998 chem. to 1 or more of 68 species of wild and domestic birds was detd. by standardized testing procedures. Red-winged blackbirds (Agelaius phoeniceus) were the most sensitive of the bird species tested on a large no. of chems., and an index based on red-wing toxicity and repellency may provide an appropriate indication of the probability of acute avian poisoning episodes. Avian repellency and toxicity were not pos. correlated (i.e., toxicity varied independently with repellency).	Rejected. The test substance was not relevant and did not require a risk assessment
Effects of 77 chemicals on reproduction in male and female coturnix quail	Seventy-one chem. were administered as single oral doses at about 50% of the estd. LD_{50} to adult male Coturnix quail (Coturnix coturnix). None reduced the fertility of eggs produced by female mates by more than 50%. Of six addnl. chem. similarly administered to female quail at 24 to 56% of the estd. LD_{50}, only one, P,P-bis(1-aziridinyl)-N-phenylphosphinic amide [6784-53-8], reduced expected egg fertility by more than 50%.	Rejected. The test substance was not relevant and did not require a risk assessment
The influence of 1,2,4-triazole and 5-oxo-1,2,4-triazyne derivatives on some blood and performance indices of turkey hens.	The experiment was carried out on 6-week-old Big-6 turkey hens receiving 1,2,4-triazole or 5-oxo-1,2,4-triazyne derivatives as additives to drinking water for ten weeks. The aim of the study was to determine the influence of the tested compounds on some immunological and haematological parameters of blood and on performance of turkey hens. The results showed that the examined substances did not significantly affect the immune response of turkey hens, although all immunological parameters in the group receiving 5-oxo-1,2,4-triazyne were slightly better than in the control group. Moreover, the 5-oxo-1,2,4-triazyne supplement caused an increase in the RBC count, Ht and Hb levels. Administration of both tested derivatives slightly improved the performance of the birds.	Rejected. The endpoint from the study, blood parameters in birds, is of no relevance to setting regulatory endpoints

Summary

Of the 15 studies classified as "hits", all bar one have been considered unsuitable. The study "The influence of anti-corrosion compounds on algal growth" has been considered further in Table B.9.11.2-5below.

As BAS 750 F is a new active substance, there is not likely to be a wealth of literature data available for this active substance. Multiple studies had hits for metabolites, but few used metabolites of BAS 750 F considered ecotoxicologically relevant according Fate and Behaviour as the test substance. Some studies were available that had tested 1,2,4-triazole, but were not suitable studies from which to derive ecotoxicological endpoints according to the relevance and reliability criteria above with the exception of the "The influence of anti-corrosion compounds on algal growth".

The literature review is considered to have suitable covered with a robust methodology and is considered acceptable, noting the issues previously highlighted.

B.9.11.2 Literature review of triazole derivatives

An additional literature review was performed on triazole derivative metabolites (1,2,4-triazole, triazole acetic acid, triazole alanine and triazole lactic acid). The search process is documented in all details with search profiles, search histories and summary tables. The review has the objective of identifying scientific peer-reviewed open literature on ecotoxicological studies which may impact health, the environment and non-target species and published within the last *ten* years before the date of submission of the dossier in accordance with Article 8(5) of Regulation (EC) No. 1107/2009, EFSA Journal 2011;9(2):2092.

Evaluation of studies

The relevance and reliability of the studies has been evaluated according to the criteria presented in Table B.9.11.2-1 below.

Table B.9.11.2-1: List of Criteria for relevance for each data requirement

Data requirements(s) (indicated by the correspondent CA data point (s))	Criteria for relevance
Ecotoxicological studies (CA 8.1 to 8.15)	Laboratory Studies 1. Well defined test material (including purity/content) 2. Number of organisms per group sufficient to establish a statistical significance 3. Applicable test species 4. Test organisms are not previously exposed to the test material or other contaminants 5. Several dose levels tested, at least 3, including a negative control, to establish a dose-response, unless the study design is specifically a limit test. Control must be run concurrently with treatments and mortality to be within test validity criteria. 6. Exposure route is clearly defined, is environmentally relevant and, if appropriate, suitably quantified. 7. If conducted, analytical confirmation of dosing or sufficient information provided to determine concentrations were within acceptable range (e.g. 80-120%) of nominal targets. 8. Effects are related to single test substance, and a quantitative relationship exists between the reported endpoint and risk assessment endpoints of growth, mortality, behaviour and/or reproduction. 9. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. 10. Study conditions should not differ significantly from recommended protocols. 11. Study conditions should not interfere with the interpretation of the study results. Field Studies 12. Appropriate and relevant geoclimatic conditions (setting), appropriate application method and rates (exposure) and observation data (biological relevance) to derive endpoints. 13. Well defined test material (including purity/content) 14. Applicable test species 15. Exposure route is clearly defined, is environmentally relevant and, if appropriate, suitably quantified. 16. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust (e.g. pre-treatment details, characterisation of physico-chemical parameters, replication, statistical methods and appropriate sampling regime). 17. Study conditions should not differ significantly from recommended protocols, if available for field study. Study conditions should not interfere with the interpretation of the study results

* Recommended protocols under each data point include but are not limited to those listed in the Commission Communications 2013/C 95/01 and 2013/C 95/02

Databases searched

Sixteen databases have been searched as part of the literature review, covering a wide range of topics. While it is noted that some may not be relevant to ecotoxicology, the number and range of databases searched is considered sufficient.

Table B.9.11.2-2: Details of Databases Searched and justification for Selection

Provider	Database	Justification	Limits applied	Number*
Host STN	MEDLINE	Contains information on every area of medicine providing comprehensive coverage from 1948 to present. Sources include journals and chapters in books or symposia. The database is updated 5 times each week with an annual reload and therefore stays very current in its cover.	None	133
	EMBASE	The database, covers worldwide literature in the biomedical and pharmaceutical fields, including biological science, biochemistry, human medicine, forensic science, pediatrics, pharmacy, pharmacology and drug therapy, pharmacoeconomics, psychiatry, public health, biomedical engineering and instrumentation, and environmental science. Sources include more than 4,000 journals from approximately 70 countries, monographs, conference proceedings, dissertations, and reports. The databases covers data from 1974-present and is updated daily.		76
	EMBAL	The database provides early access to bibliographic data and the abstracts for references that will appear in EMBASE. Bibliographic information for references is available in EMBAL for the latest 8 weeks of EMBASE data. The database covers the worldwide literature on the biomedical and pharmaceutical fields. Bibliographic information, abstracts, and author keywords are searchable. Sources include over 4,000 journals. The database covers current data and is updated daily.		0
	ESBIOBASE	A database providing comprehensive coverage of the entire spectrum of biological research worldwide. Coverage includes the following areas: applied microbiology, biotechnology, cancer research, cell & developmental biology, clinical chemistry, ecological & environmental sciences, endocrinology, genetics, immunology, infectious diseases, metabolism, molecular biology, neuroscience, plant and crop science, protein biochemistry, and toxicology. Records are selected from over 1,700 international scientific journals, books, and conference proceedings. The database covers the period 1994 - present and is updated weekly.		2
	AGRICOLA	A bibliographic database containing selected worldwide literature of agriculture and related fields. Coverage of the database includes agricultural economics and rural sociology, agricultural production, animal sciences, chemistry, entomology, food and human nutrition, forestry, natural resources, pesticides, plant science, soils and fertilizers, and water resources. Also covered are related areas such as biology and biotechnology, botany, ecology, and natural history. The database draws on bibliographies, serial articles, book chapters, monographs, computer files, serials, maps, audiovisuals, and reports. It covers the period 1970-present and is updated monthly.		2
	BIOSIS	A large and comprehensive worldwide life science database covers original research reports, reviews, and selected U.S. patents in biological and biomedical areas, with subject coverage ranging from aerospace biology to zoology. Sources include periodicals, journals, conference proceedings, reviews, reports, patents, and short communications. Nearly 6,000 life source journals, 1,500 international meetings as well as review articles, books, and monographs are reviewed for inclusion. It covers the period 1926 – present and is updated weekly.		39
	CABA	Covers worldwide literature from all areas of agriculture and related sciences including biotechnology, forestry, and veterinary medicine. Sources include journals, books, reports, published theses, conference proceedings, and patents. It covers the period 1973-present and is updated weekly.		14

Provider	Database	Justification	Limits applied	Number*
	HCAPLUS	Covers worldwide literature from all areas of chemistry, biochemistry, chemical engineering, and related sciences including applied, macromolecular, organic, physical, inorganic, and analytical chemistry. Current sources include over 8,000 journals, patents, technical reports, books, conference proceedings, dissertations, product reviews, bibliographic items, book reviews, and meeting abstracts. Electronic-only journals and Web preprints are also covered. Cited references are included for journals, conference proceedings and basic patents from the U.S., EPO, WIPO, and German patent offices added to the CAS databases from 1999 to the present. Also provides early access to the bibliographic information, abstracts and CAS Registry Numbers for documents in the process of being indexed by CAS. Covers the period 1907 – present and is updated daily		143
	FSTA	The database provides worldwide coverage of all scientific and technological aspects of the processing and manufacture of human food products including basic food sciences, biotechnology, hygiene and toxicology, engineering, packaging, and all individual foods and food products. Sources include more than 2,200 journals, books, reviews, conference proceedings, patents, standards, and legislation. It covers the period 1969 – present and is updated weekly.		0
	FROSTI	The database contains citations to the worldwide literature on food science and technology including food and beverages, analytical methods, quality control, manufacturing, microbiology, food processing, health and nutrition, recipes, and additives. Sources include approximately 800 scientific and technical journals, bulletins, technical reports, conference proceedings, grey literature, and British, European (EP), U.S., Japanese, and international (PCT) patent applications. Covers the period 1972 – present and is updated twice weekly.		0
	GEOREF	Covers international literature on geology and geosciences. Sources include the Bibliography of North American Geology, Bibliography and Index of Geology Exclusive of North America, Geophysical Abstracts, Bibliography of Fossil Vertebrates, selected records from Geoline and from geology sections of PASCAL and state and national geological surveys. Covers the period 1669 – present and is updated twice a month.		0
	TOXCENTER	Covers the pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals. It is composed of the following subfiles: BIOSIS, CAplus, IPA and MEDLINE and sources include abstracts, books and book chapters, bulletins, conference proceedings, journal articles, letters, meetings, monographs, notes, papers, patents, presentations, research and project summaries, reviews, technical reports, theses, translations, unpublished material, web reprints. Covers the period 1907 – present and is updated weekly		0
	PQSCITECH	Is a huge resource in all areas of science and technology from engineering to lifescience. The file is a merge of 25 STN databases formerly known as CSA databases (Cambridge Scientific Abstracts): AEROSPACE, ALUMINIUM, ANTE, AQUALINE, AQUASCI, BIOENG, CERAB, CIVILENG, COMPUAB, CONFSCI, COPPERLIT, CORROSION, ELCOM, EMA, ENVIROENG, HEALSAFE, LIFESCI, LISA, MATBUS, MECHENG, METADEX, OCEAN, POLLUAB, SOLIDSTATE, and WATER. Sources are journals, patents, books, reports, and conference proceedings spanning the period 1962 – present and it is updated monthly.		1

Provider	Database	Justification	Limits applied	Number*
	PASCAL	The database provides access to the world's scientific and technical literature including physics and chemistry, life sciences (biology, medicine, and psychology), applied sciences and technology, earth sciences, and information sciences. French and European literature is particularly well represented. Approximately 5,000 journal titles are indexed. References to theses and to conference proceedings are also included. Spans the period 1977 to present and is updated weekly		1
	SCISEARCH	Is an international index to the literature covering virtually every subject area within the broad fields of science, technology, and biomedicine. SciSearch contains all the records published in Science Citation Index Expanded™ and additional records from the Current Contents series of publications. Bibliographic information and cited references from over 5,600 scientific, technical, and medical journals are contained in the database. Spans the period 1974 to present and is updated weekly.		4
	ANABST	Covers worldwide literature on analytical chemistry. The ANABSTR file contains bibliographic records with abstracts (since 1984) for documents reported in printed Analytical Abstracts. Sources for ANABSTR include journals, books, conference proceedings, reports, and standards. Spans the period 1980 to present and is updated weekly.		0

* Total number of summary records retrieved after removing duplicates

Search Parameters

Initially a very broad search was done to look for any references that included the triazole common metabolites in conjunction with broad ecotoxicological terms. These covered the aforementioned databases with a date span covering the previous ten years before the date of the initial database search.

In general the terms covered a wide range of family and species names over a variety of, as well as including common test species. The search also covered reptilian and amphibian groups, and some fish. Bird species were well represented. Mammals were not only represented by Rodentia and Lagomorpha, but also some species from other orders such as otters from the Carnivora. Invertebrate species were covered by both genus level searches and higher searches covering phyla and classes. There were limited search terms for plants and algae, potentially restricting the range of studies to be found for these groups. However, in general the search terms covered a comprehensive range of ecotoxicological terms.

Table B.9.11.2-3: Detailed Search Parameters for Ecotoxicological studies (CA 8.1 to 8.15)

Dates of search	
Date of initial search	01 January 2004
Date of most recent update to search	09 June 2015
Date span of the search	2004 to 09 June 2015
Search Strategy	
L1 QUE (288-88-0 OR 1450828-63-3 OR 28711-29-7 OR 333354-47-5) L2 QUE (86362-20-1 OR 114419-45-3 OR 4819-36-7) L3 QUE (1(W)2(W)4(W)TRIAZOL(W)1(W)YLACETIC(W)ACID) L4 QUE (1(W)2(W)4(W)TRIAZOLE(W)1(W)ACETIC(W)ACID) L5 QUE (1H(2W)1(W)2(W)4(W)TRIAZOL(W)1(W)YLACETIC(W)ACID) L6 QUE (2(2W)1(W)2(W)4(W)TRIAZOL(W)1(W)YL(W)ACETIC(W)ACID) L7 QUE (2(2W)1H(2W)1(W)2(W)4(W)TRIAZOL(W)1(W)YL(W)ACETIC(W)ACID) L8 QUE ((1H(2W)1(W)2(W)4(W)TRIAZOLE) OR (S(W)TRIAZOLE)) L9 QUE ((3(W)4(W)DIAZAPYRROLE) OR	

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(4H(2W)1(W)2(W)4(W)TRIAZOLE))
L10             QUE (25167-73-1 OR 27236-77-7 OR 116421-29-5 OR 1001118-18-
8)
L11             QUE (3(2W)1H(W)1(W)2(W)4(W)TRIAZOL#(W)1(W)YL(W)ALANINE)
L12             QUE (1H(2W)1(W)2(W)4(W)TRIAZOL#(W)1(W)PROPANOIC(W)ACID)
L13             QUE (1H(2W)1(W)2(W)4(W)TRIAZOL#(W)1(W)PROPIONIC(W)ACID)
L14             QUE ((2(W)HYDROXY(W)3)(2W)(L12 OR L13))
L15             QUE ((ALPHA(W)(HYDROXY OR AMINO))(3A)(L12 OR L13))
L16             QUE (L1-11 OR L14-15)
                                    PLUS
L1              QUE  (RIPARIAN? OR REPTILE? OR SNAKE? OR LIZARD?)
L2              QUE  (TORTOISE? OR TURTLE? OR TERRAPIN? OR CROCODIL?)
L3              QUE  (ALLIGATOR? OR CAIMAN? OR GHARIAL? OR HOVERFLIES)
L4              QUE  ((MEADOW#(W)VOLE#) OR PSEUDOKIRSCHNERIELLA)
L5                     QUE   (RHAPHIDOCELIS OR NITZSCHIA OR CYCLOTELLA OR
MICROCYSTIS)
L6              QUE  (OSCILLATORIA OR APHANIZOMENON OR ANKISTRODESMUS)
L7                  QUE   (TEILINGRIA OR MONORAPHIDIUM OR RADIOCOCCACAE OR
TETRASPORALES)
L8              QUE  (TETRAEDRON OR TREUBARIA OR WILLEA OR COSMOCLADIUM)
L9               QUE  (HYPOASPIS OR (SOIL(3A)MICROORGAN?) OR ECHINOCHLOA OR
SPARTINA)
L10                 QUE   (SALVINIA OR NAJAS OR CALLITRICHE OR MYOSOTIS OR
STRATIOTES)
L11             QUE  (HIPPURUS OR PERSICARIA OR CLOEON? OR CORBICULA?)
L12             QUE  (NEOCARIDINIA? OR NEOCARIDINA? OR MYSID? OR CICHLIDAE)
L13             QUE  (CICHLID# OR LEPOMIS? OR SERRANIDAE OR PERCIFORMES)
L14             QUE  (ICTALURUS? OR POECILIA? OR ORYZIAS? OR GASTEROSTEUS?)
L15                 QUE   (GASTEROSTEIDAE OR SALVELINUS OR BRACHYDANIO? OR
CARASSIUS?)
L16             QUE  (MISGUMUS? OR CYPRINODON? OR FUNDULUS? OR MISGURNUS?)
L17                  QUE   (BREAM OR ROTIFER# OR GAMMARUS OR GAMMARID? OR
MAYFLY?)
L18             QUE  (BIVALVE# OR MUSSEL# OR MOLLUSK# OR MOLLUSC# OR BUFO)
L19                    QUE   (NEWT# OR SCALLOP# OR CLAM# OR GAMBUSIA OR
OREOCHROMIS)
L20             QUE  (OSTRAC? OR TUBIFEX? OR TURBELLARIA OR COPEPODA)
L21                 QUE   (PREDACE? OR PREDACI? OR PARASITOID? OR APIS OR
APIDAE)
L22                  QUE   (BOMBUS OR BOMBINAE OR WORM# OR LUMBRICIDAE OR
LUMBRICUS)
L23                QUE   (ALLOBOPHORA? OR DENDROBAENA? OR APORRECTODEA? OR
DENDRODRILUS?)
L24                   QUE   (EISENIA? OR OCTOLASION? OR (LACE(W)WING#) OR
NEUROPTER?)
L25              QUE  (CARABID? OR CARBUS OR STAPHYLINID? OR COCCINEL? OR
ADALIA?)
L26                QUE   (STETHORUS? OR SCYMNUS? OR WASP# OR VESPIDAE OR
SPHECOIDEA)
L27             QUE  (SPHECIDAE OR STIZIDAE OR OPIUS OR (ICHNEUMON(W)FL?))
L28               QUE  (ICHNEUMONID? OR BRACONID? OR CHALCID? OR CYNIP? OR
APHIDI?)
L29                QUE   (EUCOILID? OR IBALIID? OR FIGITID? OR EURYTOM? OR
TORYM?)
L30                QUE   (ORYM? OR EUCHARIT? OR PERILAMP? OR PTEROMAL? OR
CHRYSOLAMP?)
L31                QUE   (EUPELM? OR ENCYRT? OR SIGNIPHOR? OR APHELIN? OR
ELASMID?)
L32                  QUE   (ELASMUS OR TETRACAMP? OR MYMAR? OR HELOR? OR
PROCTOTRUP?)
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L33             QUE  (DIAPRI? OR SCELION? OR PLATYGASTR? OR PLATYGASTER?)
L34                QUE  (CERAPHRON? OR MEGASPIL? OR ARANE? OR OPILION? OR
PHALANG?)
L35                   QUE   (ARACHNID? OR HARVESTM? OR DADDYLONGLEG? OR
(DADDY(W)LONG(W)LEG?))
L36             QUE  ((DADDY(W)LONGLEG?) OR COLLEMB? OR (SPRING(W)TAIL?) OR
                     CYDNODROMUS?)
L37             QUE  (PARDOSA? OR ORIUS? OR TYPHLODROM? OR PHYTOSEIULUS? OR
SYRPHID?)
L38              QUE  (METASYRPHUS? OR SYRPHUS? OR EUPEODES? OR EPISYRPHUS?
OR SYRPHIAN?)
L39              QUE  (EPISTROPHE? OR AMBLYSEIUS? OR POECILUS? OR TRECHUS?
OR BEMBIDION?)
L40              QUE  (NEBRIA? OR PTEROSTICHUS? OR CALOSOMA? OR TACHYPORUS?
OR NABIDAE?)
L41                   QUE   (GEOCORIS? OR HYMENOPT? OR HAEMATOLOECHA? OR
CHRYSOPID? OR
                     SYMPHYTA?)
L42              QUE  (OULEMA? OR APHYTIS? OR BATHYPLECTES? OR LINPHIIDAE?
OR
                     LYNPHIIDAE?)
L43                   QUE   (LINYPHIIDAE? OR ERIGONE? OR BATHYPHANTES? OR
MEIONETA? OR
                     OEDOTHORAX?)
L44             QUE  (LEPTHYPHANTES? OR LYCOSID? OR LYCOSA? OR CHRYSOPA? OR
DACNUSA?)
L45                 QUE   (CYRTORHINUS? OR CRYPTOLAEMUS? OR ZETZELLIA? OR
LEPTOMASTIX?)
L46                  QUE   (TRICHOGRAMMA? OR ENCARSIA? OR MACROLOPHUS? OR
CHRYSOPERLA?)
L47                     QUE   (ALEOCHARA? OR CHRYSOPID# OR CHRYSOPIDAE OR
DIABROTICA)
L48               QUE   (PALEXORISTA? OR MAMMAL## OR ANIMAL? OR RABBIT? OR
RODENT#)

BIRD PROFILE   [L94]

L49                   QUE   (BLACKBIRD# OR (BLACK(W)BIRD#) OR ((TURDUS OR
T)(W)MERULA))
L50                  QUE   (CHAFFINCH? OR ((FRINGILLA OR F)(W)COELEBS) OR
GREENFINCH?)
L51             QUE  (((CARDUELIS OR C)(W)CHLORIS) OR SONGTHRUSH?)
L52              QUE  ((SONG(W)THRUSH?) OR ((TURDUS OR T)(W)PHILOMELOS) OR
WREN#)
L53                        QUE    (((TROGLODYTES  OR  T)(W)TROGLODYTES)  OR
(WILLOW(W)WARBLER#))
L54             QUE  (((PHYLLOSCOPUS OR P)(W)TROCHILUS) OR (GREAT(W)TIT#))
L55             QUE  (((PARUS OR P)(W)MAJOR) OR ROBIN# OR GOLDFINCH?)
L56             QUE  (((ERITHACUS OR E)(W)RUBECULA) OR DUNNOCK#)
L57             QUE  (((CARDUELIS OR C)(W)CARDUELIS) OR LINNET#)
L58                   QUE   (((PRUNELLA OR P)(W)MODULARIS) OR SKYLARK# OR
(SKY(W)LARK#))
L59             QUE  ((HEDGE(W)(SPARROW# OR ACCENTOR#)))
L60                  QUE   (((CARDUELIS OR C)(W)CANNABINA) OR ((ALAUDA OR
A)(W)ARVENSIS))
L61                   QUE   ((RED(W)LEGGED(W)PARTRIDGE#) OR ((ALECTORIS OR
A)(W)RUFA))
L62                QUE   ((MEADOW(W)PIPIT#) OR MEADOWPIPIT# OR ((ANTHUS OR
A)(W)PRATENSIS))
L63             QUE  (LAPWING# OR ((VANELLUS OR V)(W)VANELLUS) OR PEEWIT#)
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L64             QUE  (STARLING# OR ((STURNUS OR S)(W)VULGARIS))
L65             QUE  ((TURTLE(W)DOVE#) OR ((STREPTOPELIA OR S)(W)TURTUR))
L66                       QUE    (YELLOWHAMMER#  OR  (YELLOW(W)HAMMER#)  OR
(YELLOW(W)WAGTAIL#))
L67                            QUE     (((EMBERIZA  OR  E)(W)CITRINELLA)  OR
(YELLOW(W)WAG(W)TAIL#))
L68                               QUE     (((MOTACILLA  OR  M)(W)FLAVA)  OR
(FAN(W)TAILED(W)WARBLER#))
L69             QUE  ((GREY(W)LAG(W)G!!SE) OR ((ANSER OR A)(W)ANSER))
L70             QUE  (REEDBUNTING# OR (REED(W)BUNTING#) OR ((EMBERIZA OR E)
                     (W)SCHOENICLUS))
L71             QUE  (CHAFFINCH? OR BLUETIT? OR (BLUE(W)TIT?))
L72             QUE  (((PARUS OR P)(W)CAERULEUS) OR (SYLVIA(W)COMMUNIS))
L73             QUE  (((GALERIDA OR G)(W)CRISTATA) OR (TREE(W)SPARROW#))
L74             QUE  (((COTURNIX OR C)(W)COTURNIX) OR (GREY(W)PARTRIDGE#))
L75                    QUE   (((PERDIX  OR  P)(W)PERDIX)  OR  ((PHASIANUS  OR
P)(W)COLCHICUS))
L76             QUE  (((MILIARIA OR M)(W)CALANDRA?) OR GREYLAGG!!SE)
L77             QUE  ((GREYLAG(W)G!!SE) OR ((COLUMBA OR C)(W)PALUMBUS?))
L78             QUE  (((STREPTOPELIA OR S)(W)(ORIENTALIS? OR RISORIA?)))
L79             QUE  (((MOTACILLA OR M)(W)ALBA?) OR (CRESTED(W)LARK#))
L80                     QUE   ((WHITE(W)WAGTAIL#)  OR  (WOOD(W)PIGEON#)  OR
(BIRD(W)LIFE))
L81             QUE  ((SONG(W)BIRD#) OR VANELLUS? OR (PEE(W)WIT#))
L82             QUE  (AVIFAUNA? OR (AVI(W)FAUNA?) OR SONGBIRD?)
L83             QUE  (ORNITHOLOG? OR PASSERINE? OR WOODPIGEON#)
L84                      QUE   (((PASSER  OR  P)(W)MONTANUS)  OR  QUAIL#  OR
(CALANDRA(W)LARK#))
L85             QUE  (CISTICOLA? OR (Z(W)CISTICOLA?) OR BIRDLIFE)
L86             QUE  (GEESE OR GOOSE OR SPARROWS OR PIGEONS OR LARK#)
L87             QUE  (WARBLER# OR PARTRIDGE# OR BUNTING# OR WAGTAIL#)
L88             QUE  (WHITETHROAT# OR PIED# OR (WHITE(W)THROAT#))
L89             QUE  ((FORAGING OR FARMLAND OR GRASSLAND)(3A)BIRD#)
L90                          QUE    (BLUEBIRD#  OR  (ROCK(W)PTARMIGAN#)  OR
(BLACK(W)REDSTART#))
L91             QUE  ((PREDATOR? OR NONTARGET? OR (NON(W)TARGET))(3A)BIRD#)
L92             QUE  ((CORN(W)BUNTING#) OR SERINS OR SERINUS)
L93             QUE  (L49-L92)
L94             QUE  L93 NOT (JAPANESE? OR JAPONICA?)

MAMMALS PROFILE  [L105]

L95             QUE  (((SMALL OR WILD)(3A)MAMMAL#) OR (WILD(3A)ANIMAL?))
L96             QUE  (VOLE# OR GLIS OR DORMOUSE OR DORMICE OR ELIOMY#)
L97                  QUE   (LEROT#  OR  LAGOMORPH#  OR  LEPORID?  OR  LEPUS  OR
ORYCTOLAGUS?)
L98                    QUE   (HARE#  OR  SORICIDAE?  OR  SOREX?  OR  NEOMY#  OR
CROCIDURA?)
L99                 QUE   (SHREW# OR WOODMOUSE OR WOODMICE OR APODEMUS? OR
MICROTUS?)
L100            QUE  (CLETHRIONOMYS? OR CRICETIDAE? OR MICROTIN?)
L101            QUE  (RAPTOR# OR MARMOSET# OR GOPHER# OR GRASSCUTTER#)
L102                              QUE     ((PREDATOR?  OR  NONTARGET?  OR
(NON(W)TARGET?))(3A)MAMMAL#)
L103            QUE  ((WOOD(W)(MOUSE OR MICE)) OR ARVICOLA?)
L104            QUE  (MEADOW#(W)VOLE#)
L105            QUE  (L95-L104)

EXISTING ECOTOX PROFILE [L171]
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L106             QUE  (ECOTOX? OR LC50 OR ((LC OR EC OR LR)(W)50) OR EC50 OR
LR50)
L107                 QUE   (ECO OR ECOL OR ECOLOG? OR ENV OR ENVIRONM? OR
AQUATIC?)
L108             QUE  (L107(5A)(TOX? OR RISK? OR IMPACT? OR EFFECT?))
L109             QUE  (AQUATIC? OR FRESHWATER? OR (FRESH(W)WATER?))
L110             QUE  (FLORA OR FAUNA  OR BIOTA OR ORGANISM? OR INSECT?)
L111                 QUE   (ENVIRONM? OR LIFE OR INVERTEB? OR CRUSTACE? OR
SPECIES)
L112             QUE  (ENTOMOFAUNA OR (ENTOMO(W)FAUNA))
L113             QUE  (L109(5A)(L110 OR L111 OR L112))
L114             QUE  (MAGNA? OR (D(W)MAGNA?) OR CHIRONOM? OR BRACHIONUS?)
L115             QUE  (LIMNEA? OR CRASSOSTREA? OR ALGA# OR FISH OR FISHES)
L116                  QUE   (ONCORHYNCHUS? OR SALMONIDAE? OR CYPRINUS? OR
CYPRINID?)
L117             QUE  (PIMEPHALES? OR PISCES OR TROUT OR SUNFISH? OR CARP)
L118             QUE  (MINNOW? OR (F(W)MINNOW?) OR CATFISH? OR ZEBRAFISH?)
L119             QUE  (GOLDFISH? OR (ZEBRA(W)DANIO#) OR GUPPY OR GUPPIES)
L120             QUE  (KILLFISH? OR FATHEAD? OR BLUEGILL? OR SALMON#)
L121                  QUE   (THUNDERFISH? OR (WATER(W)(FLY OR FLEA?)) OR
WATERFLEA?)
L122              QUE  (FROG# OR AMPHIBIA? OR SHRIMP# OR PRAWN# OR CRAB# OR
TOAD#)
L123             QUE  (TADPOLE# OR CRAYFISH? OR SHELLFISH? OR LOBSTER#)
L124             QUE  (OYSTER# OR SNAIL# OR RANA OR RANIDAE? OR PLANKTON?)
L125             QUE  L106 OR L108
L126                  QUE   ((NONTARGET? OR (NON(W)TARGET?))(5A)(PLANT? OR
FLORA?))
L127             QUE  ((AQUATIC(3A)(PLANT? OR (PHYTO(W)TOX?) OR PHYTOTOX?)))
L128             QUE  (SEDIMENT? OR HYDROSOIL? OR DUCKWEED? OR PONDWEED?)
L129                    QUE   (((DUCK OR POND)(W)WEED#) OR MACROPHYT? OR
PERIPHYTON?)
L130                    QUE   (POTAMOGETON? OR CHAROPHYTA? OR ELODEA? OR
HYDROCHARITA?)
L131                 QUE   (CERATOPHYL? OR CHLAMYDOMON? OR SELENASTRUM? OR
CHLORELLA?)
L132                  QUE   (SCENEDESMUS? OR SKELETONEMA? OR NAVICULA? OR
ANABAENA?)
L133             QUE  (MYRIOPHYLLUM? OR GLYCERIA?)
L134             QUE  (NONTARGET? OR (NON(W)TARGET?) OR BENEFICIAL?)
L135               QUE  (EFFECT? OR INVERTEB? OR ORGANISM? OR ARTHROPOD? OR
INSECT?)
L136                    QUE   (FAUNA OR SPECIES OR (ENTOMO(W)FAUNA?) OR
ENTOMOFAUNA?)
L137             QUE  ((L134(5A)(L135 OR L136)))
L138                 QUE   (PREDAT? OR (NATURAL(W)ENEM?) OR BEE OR BEES OR
HONEYBEE#)
L139                   QUE   (BUMBLEBEE# OR ((HONEY OR BUMBLE)(W)BEE#) OR
EARTHWORM?)
L140                  QUE   ((EARTH(W)WORM?) OR LADYBUG# OR LADYBEETLE# OR
LADYBIRD#)
L141             QUE  ((LADY(W)(BUG# OR BEETLE# OR BIRD#)) OR HOVERFLY)
L142             QUE  (HOOVERFLIES OR SAWFLY OR SAWFLIES OR DRONEFLY)
L143             QUE  (DRONEFLIES OR FLOWERFLY OR FLOWERFLIES OR LACEWING?)
L144             QUE  (((HOVER OR DRONE OR FLOWER OR SAW)(W)(FLY OR FLIES)))
L145                   QUE   (SPIDER# OR SPRINGTAIL? OR (ROOT(W)WORM#) OR
ROOTWORM#)
L146             QUE  (L137-L145)
L147                  QUE   (BIRD? OR AVES OR AVIAN? OR (AVI(W)FAUNA?) OR
AVIFAUNA?)
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L148            QUE  (SONGBIRD? OR (SONG(W)BIRD?) OR ORNITHOLOG?)
L149            QUE  (L147 OR L148)
L150            QUE  ((WILD(3A)(LIFE OR ANIMAL#)) OR WILDLIFE OR SQUIRREL?)
L151            QUE  (VOLE# OR SCIURUS OR GLIRID? OR GLIS OR DORMOUSE)
L152            QUE  (DORMICE OR ELIOMYS OR LEROT# OR MUSTELID? OR MINK#)
L153                 QUE   (MUSTELINE# OR WEASEL? OR STOAT? OR MUSTEL? OR
BADGER?)
L154            QUE  (MELES OR MELINAE OR OTTER# OR LUTRA OR LUTRINAE)
L155               QUE   (LAGOMORPH# OR LEPORID? OR LEPUS OR ORYCTOLAGUS OR
HARE#)
L156                     QUE    (TALPA OR MOLE OR MOLES OR HEDGEHOG? OR
(HEDGE(W)HOG?))
L157                QUE   (CROCIDURA? OR SHREW# OR WOODMOUSE OR WOODMICE OR
APODEMUS)
L158                    QUE    (MICROTUS OR ARVICOLA OR CLETHRIONOMYS? OR
CRICETIDAE?)
L159            QUE  (ERINACEUS OR ERINACEIDAE? OR SORICIDAE? OR SOREX)
L160            QUE  (ENDOCRIN? OR HORMON?)
L161                QUE   (DISRUPT? OR MIMIC? OR MODULAT? OR DISORDER? OR
DISEASE?)
L162            QUE  (L160(5A)L161)
L163            QUE  (DAPHNI? OR CERIODAPHNI? OR HYALELLA? OR ASSELLUS)
L164            QUE  (L113-L124) OR L163
L165               QUE   (PHYTOPLANKTON? OR AUFWUCH# OR LEMNA? OR ARALES OR
CHARA)
L166            QUE  (L126-L129) OR (L130-L133) OR L165
L167            QUE  (NEOMYS OR MICROTINAE?)
L168            QUE  (L150-L159) OR L167
L169             QUE  (LOACH? OR STICKLEBACK? OR MUMMICHOG# OR TILAPIA? OR
ASELLUS)
L170            QUE  L164 OR L169
L171            QUE  L125 OR L170 OR L166 OR L146 OR L149 OR L168 OR L162
L172            QUE  (L1-L48)

L173            QUE  (L171 OR L172 OR L94 OR L105)  TOTAL PROFILE
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Results

Table B.9.11.2-4: Results of study selection process

Data requirement(s) captured in the search	Number (Initial Search)	Number (Top-Up Search)
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	324	91
Number of *summary records* excluded from the search results after rapid assessment for relevance	323	91
Total number of *full-text* documents assessed in detail*	1	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	1	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0

*both from bibliographic databases and other sources of peer-reviewed literature

For the initial rapid assessment, the study titles were scanned to identify whether the studies were indeed relevant to ecotoxicology or not – for example, any studies clearly not in the remit of the European review (such as studies about Brazilian species); or unambiguously belonging to other sections such as environmental fate or efficacy (the majority of the references found), were excluded. A single study was assessed in further detail, but was not considered suitable for inclusion in the

dossier, as for the reasons reported in in B.9.11-6. The RMS notes that a study not following a recommended protocol is not a reason for rejection; the point of a literature search is for the wealth of data to further inform on the risk of a substance, which it may do irrespective of following set guidelines.

Table B.9.11.2-5: List of references excluded following detailed review listed by data point number

CA data point number	Author(s)	Year	Title	Source	Reason(s) for not including the study in the dossier	Ref. ID
CA	Ambrožová, Jana; Macák, Jan	2006	The influence of anti-corrosion compounds on algal growth	Algological Studies, Volume 120, Number 1, July 2006, pp. 107-113	Does not meet criterion 1 (poorly defined test substance). No analytical verification of test substance concentrations. Study also fails to meet criteria 9 and 10, as there is insufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust, and the study did not follow a recommended protocol. The study duration was 192 hr, considerably longer than the more typical duration of 72 or 96 hr, for green algae.	310

Summary

Of the 324 summary records retrieved only one was considered for full assessment. This may indicate the criteria set out in the rapid assessment is too stringent and potentially relevant literature data may be excluded. The justification for rejecting the single study is accepted by the RMS, noting the flaws with the test substance and lack of analysis, although the lack of following a recommended protocol and increased study duration are not reasons for non-inclusion alone. However, overall, the literature review has a robust methodology and is considered acceptable, noting the issues highlighted above.

B.9.12. QSAR DATA

Acute fish testing was not performed for M750F003, M750F005 and M750F008, however the applicant has undertaken QSAR modelling to define endpoints for these metabolites. The RMS has confirmed the smiles notation used. It is also noted that the applicant QSAR model was ran using v1.0, the RMS has confirmed the applicant's output from this model as correct, but has also ran a QSAR model using the updated version of ECOSAR, v1.1 (as part of Episuite 4.1). The output from the v1.1 models have been presented for each metabolite below and been assessed by the RMS according to the following approach:

- Ensure the compound is not in the training set
- Check that the log K_{OW} is within the appropriate range
- Consider the actual outputs in terms of the number of compounds in a training set and the R^2 value. In doing this, an endpoint is considered more reliable if it has a larger number of compounds in the training set and an R^2 value close to 1. However, where this is not the case, the lowest endpoint – regardless of n or R^2 has been used. The logic here is that although the endpoint, in itself, may not be entirely reliable, it is considered sufficiently protective, as it is lower than endpoints that are more reliable.

M750F003

Data on *Daphnia* and algae have been submitted that indicate decreased toxicity in comparison to the active substance. The QSAR submitted by the applicant for M750F003 was considered acceptable.

The applicant's QSAR was identical to the RMS' v.1.0, results. However, the more recently updated v.1.1 QSAR results have been presented below.

ECOSAR Version 1.11 Results Page

SMILES : CC(Cn1cncn1)(c2ccc(cc2C(F)(F)F)O)O
CHEM :
CAS Num:
ChemID1:
MOL FOR: C12 H12 F3 N3 O2
MOL WT : 287.24
Log K_{OW}: 1.383 (EPISuite K_{OW}win v1.68 Estimate)
Log K_{OW}: 0.410 (User Entered)
Log K_{OW}: (PhysProp DB exp value - for comparison only)
Melt Pt: (User Entered for Wat Sol estimate)
Melt Pt: (deg C, PhysProp DB exp value for Wat Sol estimate)
Wat Sol: 7.998E+004 (mg/L, EPISuite WSK_{OW}win v1.43 Estimate)
Wat Sol: 2460 (mg/L, User Entered)
Wat Sol: (PhysProp DB exp value)

Values used to Generate ECOSAR Profile

Log K_{OW}: 0.410 (User Entered)
Wat Sol: 2460 (mg/L, User Entered)

ECOSAR v1.11 Class-specific Estimations

Phenols
Benzyl Alcohols
Triazoles (Non-Fused)

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)	Flag
Phenols	Fish	96-hr	LC_{50}	806.508	
Phenols	Daphnid	48-hr	LC_{50}	119.62	
Phenols	Green Algae	96-hr	EC_{50}	642.097	
Phenols	Fish		ChV	64.504	
Phenols	Daphnid		ChV	22.815	
Phenols	Green Algae		ChV	307.784	
Phenols	Fish (SW)	96-hr	LC_{50}	486.865	
Phenols	Earthworm	14-day	LC_{50}	1192.342	
Phenols	Lemna gibba	7-day	EC_{50}	856.306	
Benzyl Alcohols	Fish	96-hr	LC_{50}	2192.087	
Benzyl Alcohols	Daphnid	48-hr	LC_{50}	1534.338	
Benzyl Alcohols	Green Algae	96-hr	EC_{50}	363.74	
Benzyl Alcohols	Fish		ChV	138.042	!
Benzyl Alcohols	Daphnid		ChV	170.977	!
Benzyl Alcohols	Green Algae		ChV	120.4	
Triazoles (Non-Fused)	Fish	96-hr	LC_{50}	1619.768	
Triazoles (Non-Fused)	Daphnid	48-hr	LC_{50}	68.673	

Triazoles (Non-Fused)	Green Algae	96-hr	EC_{50}	75.987	
Triazoles (Non-Fused)	Fish		ChV	2.013	
Triazoles (Non-Fused)	Daphnid		ChV	17.928	
Triazoles (Non-Fused)	Green Algae		ChV	60.025	
Triazoles (Non-Fused)	Fish (SW)	96-hr	LC_{50}	5859.761	*
Triazoles (Non-Fused)	Mysid (SW)	96-hr	LC_{50}	545.812	
Triazoles (Non-Fused)	Fish (SW)		ChV	0.693	
Triazoles (Non-Fused)	Mysid (SW)		ChV	11770.227	*

Neutral Organic (Baseline Toxicity)	SAR :	Fish		96-hr	LC_{50}	6321.628	*
	:	Daphnid	48-hr	LC_{50}	3099.344	*	
	:	Green Algae	96-hr	EC_{50}	1258.195		
	:	Fish		ChV	519.663		
	:	Daphnid		ChV	200.875		
	:	Green Algae		ChV	237.608		

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

NOTE: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu.

Class Specific $LogK_{OW}$ Cut-Offs

If the log K_{OW} of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Phenols:

Maximum $LogK_{OW}$: 7.0 (Fish 96-hr LC_{50}, Daphnid LC_{50})
Maximum $LogK_{OW}$: 6.4 (Earthworm, Lemna)
Maximum $LogK_{OW}$: 7.0 (Green Algae EC_{50}
Maximum $LogK_{OW}$: 8.0 (ChV)
Maximum $LogK_{OW}$: 5.0 (Fish (SW) 96-hr LC_{50}, Mysid)

Benzyl Alcohols:

Maximum $LogK_{OW}$: 5.8 (Fish LC_{50})
Maximum $LogK_{OW}$: 5.0 (Daphnid LC_{50})
Maximum $LogK_{OW}$: 6.4 (Green Algae EC_{50})
Maximum $LogK_{OW}$: 8.0 (Chronic Values)

Triazoles (Non-Fused):

Maximum $LogK_{OW}$: 5.0 (LC_{50})
Maximum $LogK_{OW}$: 6.4 (EC_{50})
Maximum $LogK_{OW}$: 8.0 (ChV)

Baseline Toxicity SAR Limitations:

Maximum LogK_{OW}: 5.0 (Fish 96-hr LC_{50}; Daphnid LC_{50})
Maximum LogK_{OW}: 6.4 (Green Algae EC_{50})
Maximum LogK_{OW}: 8.0 (ChV)

M750F003 was not in the QSAR training set for ECOSAR v1.11.

As the toxicity to fish is being investigated, only the predicted toxicities for fish have been considered for use as endpoints, although the suitability of the predicted endpoints will be checked using measured data for algae and *Daphnia* and comparing it to the predicted values.

Only those predicted toxicity endpoints for timescales that are consistent with guideline study timescales and are endpoints used in the aquatic risk assessment, have been considered further. Predicted acute toxicity endpoints will be considered, chronic toxicity studies are not required as there is no indication of the metabolites being more toxic than the active substance.

It is also noted that saltwater species endpoints are stated for some compound structures. Where these saltwater endpoints are more sensitive than the overall fish endpoint, the saltwater endpoint has been used as that endpoint is protective of fish.

The endpoints considered further are stated in Table B.9.2-13 below:

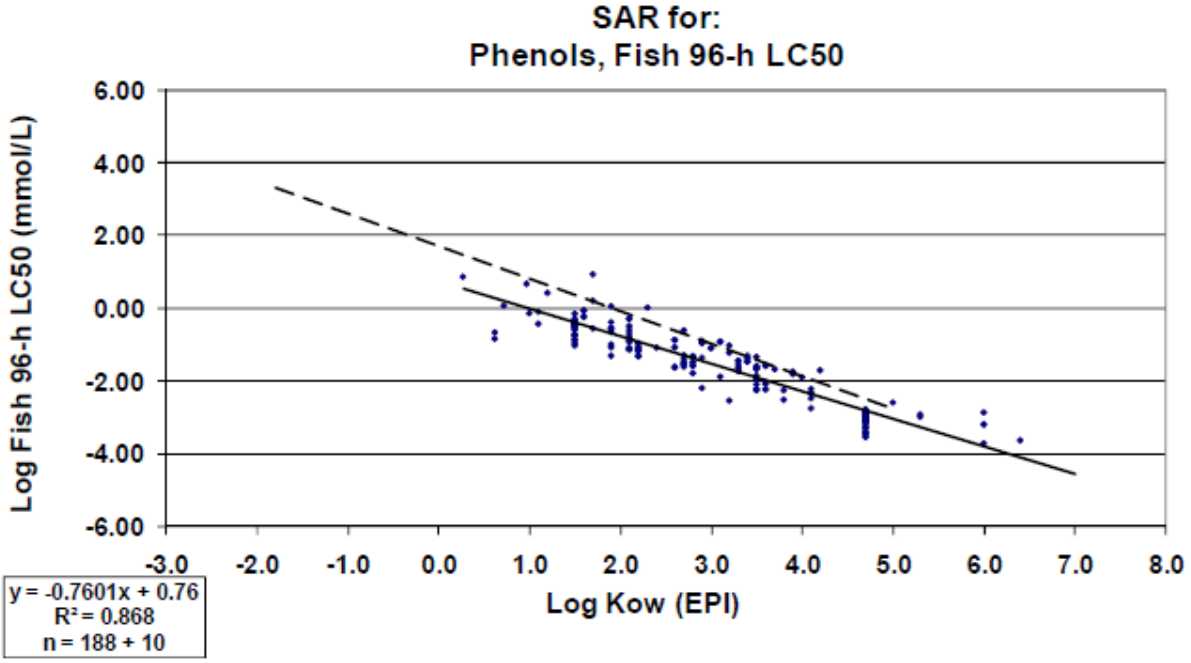
Table B.9.2-23: QSAR endpoints for M750F003 by chemical class

Chemical class	Organism	Duration	Endpoint (mg/L)	R^2	Number
Phenols	Fish	96 hr LC_{50}	486.865	0.868	188+10
Benzyl Alcohols			2192.087	0.9312	11+4
Triazoles (Non-Fused)			1619.768	0.7088	21+8

The RMS has further considered the data behind the SAR's for each aquatic organisms group for each compound structure. SAR limitations for the compound structure groups are also stated on the ECOSAR model output. These limitations are also in regards to chemicals that may result in 'no effects at saturation' during a 48 hour to 96 hour test. These limitations are dependent on the Log K_{OW} and the molecular weight of the metabolite. For ECOSAR v1.11, the predicted Log K_{OW} of M750F003 is 1.383 and the molecular weight is 287.24. The limitations have been considered further for each compound structure group, together with any additional limitations that the RMS has identified.

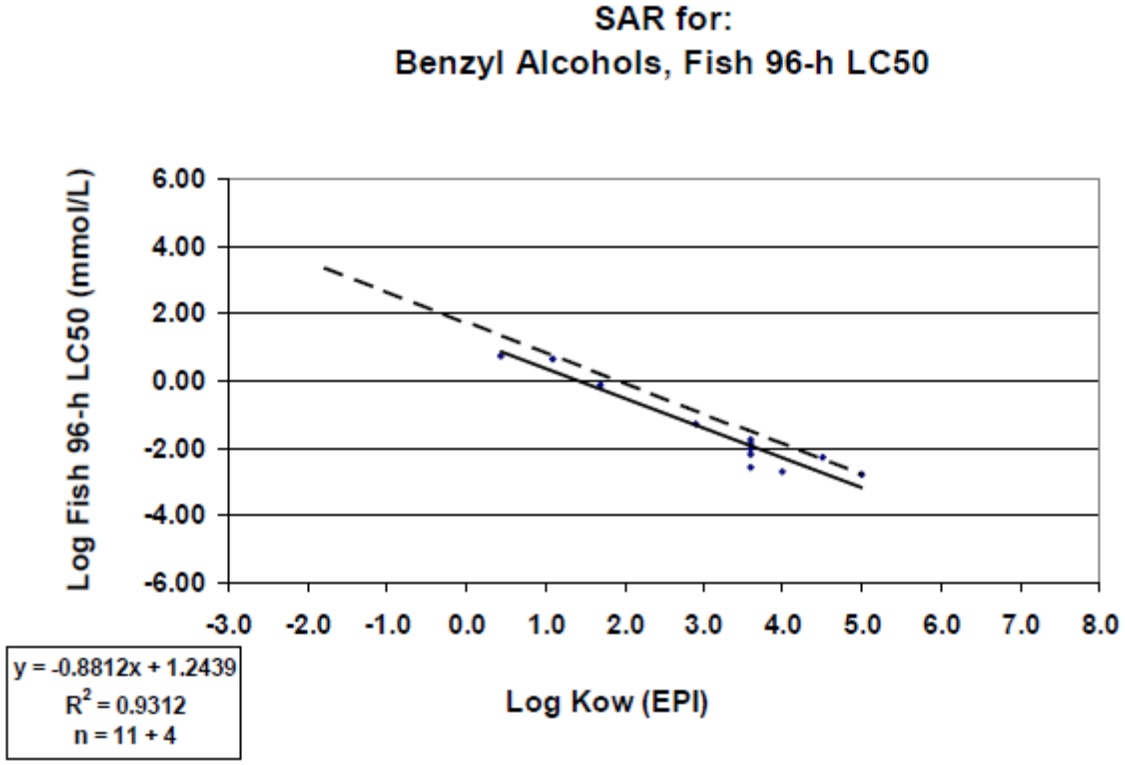
Phenols

The linear regression for toxicity to fish (96 hour LC_{50}) is based on a large dataset of 188, with a coefficient of determination (R^2) of 0.868. The recommended maximum K_{OW} is 7.0 and the maximum molecular weight (MW) is 1000. Based on the large dataset and the R^2 value, the RMS considered the SAR reliable. It is also noted that the log K_{OW} of 0.410 falls within the range of the dataset and the molecular weight is within the maximum stated for this dataset.



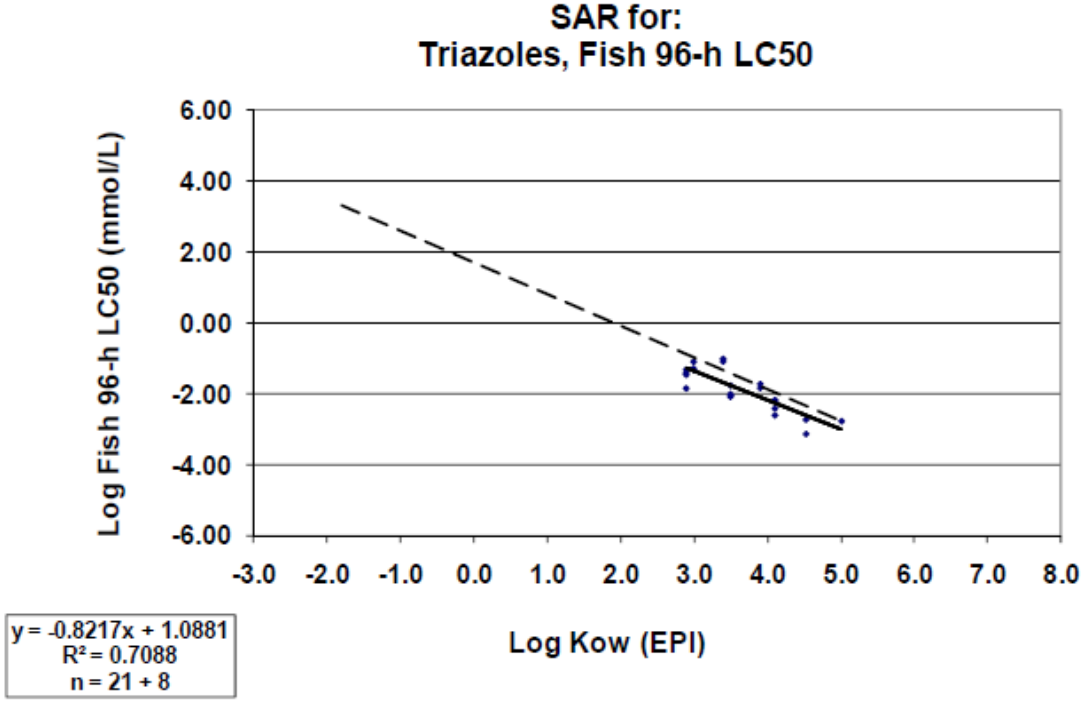
Benzyl Alcohols

The linear regression for toxicity to fish (96 hour LC_{50}) is based on a small dataset of 11, with a coefficient of determination (R^2) of 0.9312. The recommended maximum K_{OW} is 5.0 and the maximum molecular weight (MW) is 1000. Although the dataset is relatively small, due the high R2 value, the RMS considered the SAR reliable. It is also noted that the log K_{OW} of 0.410 falls within the range of the dataset and the molecular weight is within the maximum stated for this dataset.



Triazoles (Non-Fused)

The linear regression for toxicity to fish (96 hour LC_{50}) is based on a dataset of 21, with a coefficient of determination (R^2) of 0.7088. The recommended maximum K_{OW} is 5.0 and the maximum molecular weight (MW) is 1000. The dataset is somewhat small and a lower R^2 in comparison to the other classes, although there is good visual fit of the data points. However the log K_{OW} of 0.410 is less than the smallest log K_{OW} of the training dataset (2.9) and therefore the predicted toxicity for M750F003 represents an extrapolation, and as the dataset is small, the endpoint is not as reliable as the other chemical classes



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Considerations for *Daphnia* and algae endpoints

While the QSAR endpoints for *Daphnia* and algae will not be used in support of the risk assessments, they will be compared to the measured toxicity values to add further evidence to evaluating the QSAR endpoints for fish. Therefore the SARs for *Daphnia* and algae have also been considered.

The endpoint for non-fused triazoles is considered less reliable for Daphnia and algae than the other chemical classes for many of the same reasons as for fish. For *Daphnia* the non-fused triazoles training dataset consisted of only 9 data points and a low R^2 value of 0.5454 and the lowest log K_{OW} was 2.9 meaning the endpoint had to be extrapolated. For algae the dataset was smaller (8 data points) but had a higher R^2 value of 0.7702, although again the minimum log K_{OW} was 2.9 and the endpoint had to be extrapolated. However as these endpoints are the most sensitive, they will be used; while the endpoint itself may not be suitably reliable, it is considered sufficiently protective as it is lower than potentially more reliable endpoints.

Conclusion

The most sensitive acute QSAR endpoint for fish is 486.865 mg/L for phenols, and the SAR is considered reliable for this metabolite. This value is supported by the measured *Daphnia* and algae endpoints for M750F003 being in line with the values predicted by the QSAR, and the measured values were >100 mg/L for both groups. As the proposed endpoint is greater than 100 mg/L, the endpoint is being set to >100 mg/L because compounds of such low toxicity are not included in training datasets by standard, and OECD study guidelines specify limit tests of 100 mg/L. As algae and *Daphnia* measured endpoints for M750F003, the fish endpoint for other chemically related

metabolites and the QSAR endpoint of M750F003 for fish were less toxic than the parent, it can be concluded that M750F003 is not expected to be more toxic than the active substance.

Table B.9.2-24: Comparison of endpoints available for M750F003

Substance	QSAR predicted endpoints (mg/L)		
	Fish acute (96-hours)	*Daphnia* acute (48-hours)	Algae (96 hours)
M750F003 (QSAR predicted)	>100	68.673	75.987
M750F003 (measured)	-	>100	>100
BAS 750 F (measured)	0.532	0.944	0.679

M750F005

Data on *Daphnia* and algae have been submitted that indicate decreased toxicity in comparison to the active substance. The QSAR submitted by the applicant for M750F005 was considered acceptable. The applicant's QSAR was identical to the RMS' v.1.0, results. However, the more recently updated v.1.1 QSAR results have been presented below.

ECOSAR Version 1.11 Results Page

SMILES : CC(Cn1cncn1)(c2ccc(cc2C(F)(F)F)Oc3ccc(cc3)O)O
CHEM :
CAS Num:
ChemID1:
MOL FOR: C18 H16 F3 N3 O3
MOL WT : 379.34
Log K_{OW}: 3.439 (EPISuite K_{OW}win v1.68 Estimate)
Log K_{OW}: 1.690 (User Entered)
Log K_{OW}: (PhysProp DB exp value - for comparison only)
Melt Pt: (User Entered for Wat Sol estimate)
Melt Pt: (deg C, PhysProp DB exp value for Wat Sol estimate)
Wat Sol: 1820 (mg/L, EPISuite WSK_{OW}win v1.43 Estimate)
Wat Sol: 11.3 (mg/L, User Entered)
Wat Sol: (PhysProp DB exp value)

Values used to Generate ECOSAR Profile

Log K_{OW}: 1.690 (User Entered)
Wat Sol: 11.3 (mg/L, User Entered)

ECOSAR v1.11 Class-specific Estimations

Phenols
Benzyl Alcohols
Triazoles (Non-Fused)

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)	Flag
Phenols	Fish	96-hr	LC_{50}	113.36	*
Phenols	Daphnid	48-hr	LC_{50}	29.731	*

Phenols	Green Algae	96-hr	EC_{50}	140.925	*
Phenols	Fish		ChV	10.936	
Phenols	Daphnid		ChV	5.659	
Phenols	Green Algae		ChV	66.465	*
Phenols	Fish (SW)	96-hr	LC_{50}	54.058	*
Phenols	Earthworm	14-day	LC_{50}	471.834	*
Phenols	Lemna gibba	7-day	EC_{50}	87.547	*
Benzyl Alcohols	Fish	96-hr	LC_{50}	215.626	*
Benzyl Alcohols	Daphnid	48-hr	LC_{50}	166.982	*
Benzyl Alcohols	Green Algae	96-hr	EC_{50}	64.759	*
Benzyl Alcohols	Fish		ChV	17.876	*!
Benzyl Alcohols	Daphnid		ChV	25.718	*!
Benzyl Alcohols	Green Algae		ChV	30.146	*
Triazoles (Non-Fused)	Fish	96-hr	LC_{50}	189.87	*
Triazoles (Non-Fused)	Daphnid	48-hr	LC_{50}	27.191	*
Triazoles (Non-Fused)	Green Algae	96-hr	EC_{50}	20.14	*
Triazoles (Non-Fused)	Fish		ChV	0.449	
Triazoles (Non-Fused)	Daphnid		ChV	3.843	
Triazoles (Non-Fused)	Green Algae		ChV	17.02	*
Triazoles (Non-Fused)	Fish (SW)	96-hr	LC_{50}	601.035	*
Triazoles (Non-Fused)	Mysid (SW)	96-hr	LC_{50}	58.394	*
Triazoles (Non-Fused)	Fish (SW)		ChV	0.406	
Triazoles (Non-Fused)	Mysid (SW)		ChV	246.176	*

Neutral Organic SAR	: Fish	96-hr	LC_{50}	591.618 *
(Baseline Toxicity)	: Daphnid	48-hr	LC_{50}	326.445 *
	: Green Algae	96-hr	EC_{50}	216.029 *
	: Fish		ChV	55.909 *
	: Daphnid		ChV	29.398 *
	: Green Algae		ChV	53.080 *

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

NOTE: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu.

Class Specific LogK_{OW} Cut-Offs

If the log K_{OW} of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Phenols:

Maximum LogK_{OW}: 7.0 (Fish 96-hr LC_{50}, Daphnid LC_{50})

Maximum LogK_{OW}: 6.4 (Earthworm, Lemna)
Maximum LogK_{OW}: 7.0 (Green Algae EC_{50}
Maximum LogK_{OW}: 8.0 (ChV)
Maximum LogK_{OW}: 5.0 (Fish (SW) 96-hr LC_{50}, Mysid)

Benzyl Alcohols:

Maximum LogK_{OW}: 5.8 (Fish LC_{50})
Maximum LogK_{OW}: 5.0 (Daphnid LC_{50})
Maximum LogK_{OW}: 6.4 (Green Algae EC_{50})
Maximum LogK_{OW}: 8.0 (Chronic Values)

Triazoles (Non-Fused):

Maximum LogK_{OW}: 5.0 (LC_{50})
Maximum LogK_{OW}: 6.4 (EC_{50})
Maximum LogK_{OW}: 8.0 (ChV)

Baseline Toxicity SAR Limitations:

Maximum LogK_{OW}: 5.0 (Fish 96-hr LC_{50}; Daphnid LC_{50})
Maximum LogK_{OW}: 6.4 (Green Algae EC_{50})
Maximum LogK_{OW}: 8.0 (ChV)

As the toxicity to fish is being investigated, only the predicted toxicities for fish have been considered for use as endpoints, although the suitability of the predicted endpoints will be checked using measured data for algae and *Daphnia* and comparing it to the predicted values.

Only those predicted toxicity endpoints for timescales that are consistent with guideline study timescales and are endpoints used in the aquatic risk assessment, have been considered further. Predicted acute toxicity endpoints will be considered, chronic toxicity studies are not required as there is no indication of the metabolites being more toxic than the active substance.

It is also noted that saltwater species endpoints are stated for some compound structures. It is also noted that saltwater species endpoints are stated for some compound structures. Where these saltwater endpoints are more sensitive than the overall fish endpoint, the saltwater endpoint has been used as that endpoint is protective of fish.

The endpoints which are considered further are stated in the Table B.9.2-14 below. It is noted that due to the solubility of M750F005, many of the endpoints are greater than the solubility of the metabolite. The ECOSAR decision scheme states a factor of $\geq$ 10 times above the water solubility in order for an effect endpoint to be considered a low concern. The endpoints for benzyl alcohols and non-fused triazoles were at least 10 times greater than the solubility. Therefore, the endpoints were considered to have no effects at saturation, and therefore should be classified as of low concern. The endpoint for phenol is 4.8 times the solubility and so may have no effects at saturation, or effects might occur. Given this, the endpoint should be set to the saturation concentration rather than a greater than the solubility concentration.

Table B.9.2-25: QSAR endpoints for M750F005 by chemical class

Chemical class	Organism	Duration	Endpoint (mg/L)	R^2	Number
Phenols	Fish	96 hr LC_{50}	54.058	0.868	188+10
Benzyl Alcohols			215.626	0.9312	11+4
Triazoles (Non-Fused)			189.87	0.7088	21+8

The RMS has further considered the data behind the SAR's for each aquatic organisms group for each compound structure.

SAR limitations for the compound structure groups are also stated on the ECOSAR model output. These limitations are also in regards to chemicals which may result in 'no effects at saturation' during a 48 hour to 96 hour test. These limitations are dependent on the Log K_{OW} and the molecular weight of the metabolite. For ECOSAR v1.11, the measured Log K_{OW} for M750F003 is 1.69 and the molecular weight is 379.34. The limitations have been considered further for each compound structure group, together with any additional limitations that the RMS has identified.

Phenols

The SAR for phenols has previously been considered reliable under the evaluation of the M750F003 QSAR. The log K_{OW} of 1.690 falls within the range of the dataset and the molecular weight is within the recommended maximum. Therefore the phenol endpoint for M750F005 is considered acceptable.

Benzyl alcohols

The SAR for benzyl alcohols has previously been considered reliable under the evaluation of the M750F003 QSAR. The log K_{OW} of 1.690 falls within the range of the dataset and the molecular weight is within the recommended maximum. Therefore the benzyl alcohol endpoint for M750F005 is considered acceptable.

Triazoles (non-fused)

The SAR for non-fused triazoles has previously been considered under the evaluation of the M750F003 QSAR. However the log K_{OW} for M750F005 of 1.69 does not fall within the range of log K_{OW} values used in the training dataset (minimum 2.9). Consequently, there is some uncertainty over the predicted toxicity as it is an extrapolated value from a small dataset, the endpoint is not as reliable as the other chemical classes.

Considerations for *Daphnia* and algae endpoints

While the QSAR endpoints for *Daphnia* and algae will not be used in support of the risk assessments, they will be compared to the measured toxicity values to add further evidence to evaluating the QSAR endpoints for fish. Therefore the SARs for *Daphnia* and algae have also been considered.

The endpoint for non-fused triazoles is considered less reliable for Daphnia and algae than the other chemical classes for many of the same reasons as for fish. For *Daphnia* the non-fused triazoles training dataset consisted of only 9 data points and a low R^2 value of 0.5454 and the lowest log K_{OW} was 2.9 meaning the endpoint had to be extrapolated. For algae the dataset was smaller (8 data points) but had a higher R^2 value of 0.7702, although again the minimum log K_{OW} was 2.9 and the endpoint had to be extrapolated. However as these endpoints are the most sensitive, they will be used; while the endpoint itself may not be suitably reliable, it is considered sufficiently protective as it is lower than potentially more reliable endpoints.

Conclusion

The most sensitive acute QSAR endpoint for fish is 54.058 mg/L for phenols, and the SAR is considered reliable for this metabolite. Due to the solubility of 11.4 mg/L for M750F005 (III CA B.2.14), the predicted toxicities exceed the measured solubility for this metabolite, the solubility of the metabolite itself should be used as a surrogate endpoint. This has been done for fish, *Daphnia* and algae. The predicted toxicity for fish is supported by the measured *Daphnia* and algae endpoints for M750F005 being in line with the values predicted by the QSAR, although it is difficult to verify this due to the low solubility of M750F005 resulting in greater than measured values. As algae and

Daphnia measured endpoints for M750F005, the fish endpoint for other chemically related metabolites and the QSAR endpoint of M750F005 for fish were less toxic than the parent, it can be concluded that M750F005 is not expected to be more toxic than the active substance.

Table B.9.2-26: Comparison of endpoints available for M750F005

Substance	QSAR predicted endpoints (mg/L)		
	Fish acute (96-hours)	*Daphnia* acute (48-hours)	Algae (96 hours)
M750F005 (QSAR predicted)	11.3[1]	>11.3[1]	>11.3[1]
M750F005 (measured)	-	>8.58	>8.57
BAS 750 F (measured)	0.532	0.944	0.679

[1] Adjusted to surrogate toxicity based on the maximum measured solubility of the metabolite

M750F008

Data on *Daphnia* and algae have been submitted that indicate decreased toxicity in comparison to the active substance. The QSAR submitted by the applicant for M750F005 was considered acceptable. The notifier's QSAR was identical to the RMS' v.1.0, results. However the more recently updated v.1.1 QSAR results have been presented below.

ECOSAR Version 1.11 Results Page

SMILES : CC1(c2ccc(cc2C(=O)O1)c3cc(ccc3O)Cl)Cn4cncn4
CHEM :
CAS Num:
ChemID1:
MOL FOR: C18 H14 CL1 N3 O3
MOL WT : 355.78
Log K_{OW}: 2.467 (EPISuite K_{OW}win v1.68 Estimate)
Log K_{OW}: 1.760 (User Entered)
Log K_{OW}: (PhysProp DB exp value - for comparison only)
Melt Pt: (User Entered for Wat Sol estimate)
Melt Pt: (deg C, PhysProp DB exp value for Wat Sol estimate)
Wat Sol: 682.4 (mg/L, EPISuite WSK_{OW}win v1.43 Estimate)
Wat Sol: 1.96 (mg/L, User Entered)
Wat Sol: (PhysProp DB exp value)

Values used to Generate ECOSAR Profile

Log K_{OW}: 1.760 (User Entered)
Wat Sol: 1.96 (mg/L, User Entered)

ECOSAR v1.11 Class-specific Estimations

Esters
Phenols

Triazoles (Non-Fused)

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)	Flag
Esters	Fish	96-hr	LC_{50}	65.682	*
Esters	Daphnid	48-hr	LC_{50}	140.089	*
Esters	Green Algae	96-hr	EC_{50}	61.932	*
Esters	Fish		ChV	5.196	*
Esters	Daphnid		ChV	102.632	*
Esters	Green Algae		ChV	14.685	*
Esters	Fish (SW)	96-hr	LC_{50}	101.398	*
Esters	Mysid	96-hr	LC_{50}	112.251	*
Esters	Fish (SW)		ChV	13.921	*
Esters	Mysid (SW)		ChV	13471.41	*
Esters	Earthworm	14-day	LC_{50}	5543.21	*
Phenols	Fish	96-hr	LC_{50}	94.061	*
Phenols	Daphnid	48-hr	LC_{50}	25.45	*
Phenols	Green Algae	96-hr	EC_{50}	119.818	*
Phenols	Fish		ChV	9.167	*
Phenols	Daphnid		ChV	4.844	*
Phenols	Green Algae		ChV	56.46	*
Phenols	Fish (SW)	96-hr	LC_{50}	44.28	*
Phenols	Earthworm	14-day	LC_{50}	414.306	*
Phenols	Lemna gibba	7-day	EC_{50}	71.389	*
Triazoles (Non-Fused)	Fish	96-hr	LC_{50}	155.989	*
Triazoles (Non-Fused)	Daphnid	48-hr	LC_{50}	23.877	*
Triazoles (Non-Fused)	Green Algae	96-hr	EC_{50}	17.301	*
Triazoles (Non-Fused)	Fish		ChV	0.382	
Triazoles (Non-Fused)	Daphnid		ChV	3.263	*
Triazoles (Non-Fused)	Green Algae		ChV	14.675	*
Triazoles (Non-Fused)	Fish (SW)	96-hr	LC_{50}	490.191	*
Triazoles (Non-Fused)	Mysid (SW)	96-hr	LC_{50}	47.735	*
Triazoles (Non-Fused)	Fish (SW)		ChV	0.364	
Triazoles (Non-Fused)	Mysid (SW)		ChV	184.054	*

Neutral Organic SAR (Baseline Toxicity)	: Fish	96-hr	LC_{50}	591.618 *
	: Daphnid	48-hr	LC_{50}	326.445 *
	: Green Algae	96-hr	EC_{50}	216.029 *
	: Fish	ChV		55.909 *
	: Daphnid	ChV		29.398 *
	: Green Algae	ChV		53.080 *

Note: * = asterisk designates: Chemical may not be soluble enough to
/ measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

NOTE: ! = exclamation designates: The toxicity value was estimated through application of acute-to-chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu.

Class Specific LogK_{OW} Cut-Offs

If the log K_{OW} of the chemical is greater than the endpoint specific cut-offs presented below, then no effects at saturation are expected for those endpoints.

Phenols:

Maximum LogK_{OW}: 7.0 (Fish 96-hr LC_{50}, Daphnid LC_{50})
Maximum LogK_{OW}: 6.4 (Earthworm, Lemna)
Maximum LogK_{OW}: 7.0 (Green Algae EC_{50}
Maximum LogK_{OW}: 8.0 (ChV)
Maximum LogK_{OW}: 5.0 (Fish (SW) 96-hr LC_{50}, Mysid)

Benzyl Alcohols:

Maximum LogK_{OW}: 5.8 (Fish LC_{50})
Maximum LogK_{OW}: 5.0 (Daphnid LC_{50})
Maximum LogK_{OW}: 6.4 (Green Algae EC_{50})
Maximum LogK_{OW}: 8.0 (Chronic Values)

Triazoles (Non-Fused):

Maximum LogK_{OW}: 5.0 (LC_{50})
Maximum LogK_{OW}: 6.4 (EC_{50})
Maximum LogK_{OW}: 8.0 (ChV)

Baseline Toxicity SAR Limitations:

Maximum LogK_{OW}: 5.0 (Fish 96-hr LC_{50}; Daphnid LC_{50})
Maximum LogK_{OW}: 6.4 (Green Algae EC_{50})
Maximum LogK_{OW}: 8.0 (ChV)

As the toxicity to fish is being investigated, only the predicted toxicities for fish have been considered for use as endpoints, although the suitability of the predicted endpoints will be checked using measured data for algae and *Daphnia* and comparing it to the predicted values.

Only those predicted toxicity endpoints for timescales that are consistent with guideline study timescales and are endpoints used in the aquatic risk assessment, have been considered further. Predicted acute toxicity endpoints will be considered, chronic toxicity studies are not required as there is no indication of the metabolites being more toxic than the active substance.

It is also noted that saltwater species endpoints are stated for some compound structures. It is also noted that saltwater species endpoints are stated for some compound structures. Where these saltwater endpoints are more sensitive than the overall fish endpoint, the saltwater endpoint has been used as that endpoint is protective of fish.

The endpoints which are considered further are stated in Table B.9.2-26 below. It is noted that due to the solubility of M750F005, many of the endpoints are greater than the solubility of the metabolite. The ECOSAR decision scheme states a factor of ≥ 10 times above the water solubility in order for an effect endpoint to be considered a low concern. All of the endpoints listed were at least 10 times greater than the solubility. Therefore, the endpoints were considered to have no effects at saturation, and therefore should be classified as of low concern.

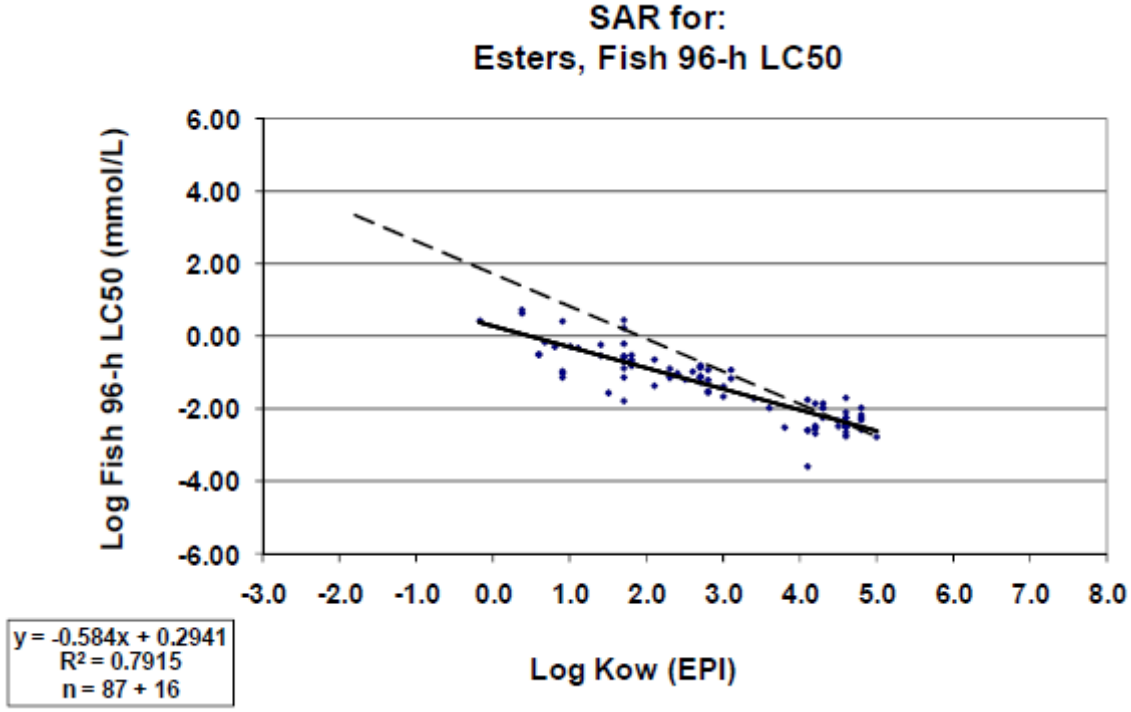
Table B.9.2-27: QSAR endpoints for M750F008 by chemical class

Chemical class	Organism	Duration	Endpoint (mg/L)	R^2	Number
Esters	Fish	96 hr LC_{50}	65.682	0.7915	87+16
Phenols			44.28	0.868	188+10
Triazoles (Non-Fused)			155.989	0.7088	21+8

The RMS has further considered the data behind the SAR's for each aquatic organisms group for each compound structure. SAR limitations for the compound structure groups are also stated on the ECOSAR model output. These limitations are also in regards to chemicals which may result in 'no effects at saturation' during a 48 hour to 96 hour test. These limitations are dependent on the Log K_{OW} and the molecular weight of the metabolite. For ECOSAR v1.11, the predicted Log K_{OW} of M750F008 is 1.76 and the molecular weight is 355.78. The limitations have been considered further for each compound structure group, together with any additional limitations that the RMS has identified.

Esters

The linear regression for toxicity to fish (96 hour LC_{50}) is based on a large dataset of 87, with a coefficient of determination (R^2) of 0.7915. The recommended maximum K_{OW} is 5.0 and the maximum molecular weight (MW) is 1000. Based on the large dataset and the R2 value, the RMS considered the SAR reliable. It is also noted that the log K_{OW} of 1.760 falls within the range of the dataset and the molecular weight is within the maximum stated for this dataset. Therefore the ester endpoint for M750F008 is considered acceptable.



Phenols

The SAR for phenols has previously been considered reliable under the evaluation of the M750F003 QSAR. The log K_{OW} of falls within the range of the dataset and the molecular weight is within the recommended maximum. Therefore the phenol endpoint for M750F008 is considered acceptable.

Triazoles (non-fused)

The SAR for non-fused triazoles has previously been considered reliable under the evaluation of the M750F003 QSAR. However the log K_{OW} for M750F008 of 1.76 does not fall within the range of log K_{OW} values used in the training dataset (minimum 2.9). Consequently, there is some uncertainty over the predicted toxicity as it is an extrapolated value and the training dataset is small, the endpoint is not as reliable as the other chemical classes.

Considerations for *Daphnia* and algae endpoints

While the QSAR endpoints for *Daphnia* and algae will not be used in support of the risk assessments, they will be compared to the measured toxicity values to add further evidence to evaluating the QSAR endpoints for fish. Therefore the SARs for *Daphnia* and algae have also been considered.

The endpoint for non-fused triazoles is considered to be less reliable for Daphnia and algae than the other chemical classes for many of the same reasons as for fish. For *Daphnia* the non-fused triazoles training dataset consisted of only 9 data points and a low R^2 value of 0.5454 and the lowest log K_{OW} was 2.9 meaning the endpoint had to be extrapolated. For algae the dataset was smaller (8 data points) but had a higher R^2 value of 0.7702, although again the minimum log K_{OW} was 2.9 and the endpoint had to be extrapolated. However as these endpoints are the most sensitive, they will be used; while the endpoint itself may not be suitably reliable, it is considered sufficiently protective as it is lower than potentially more reliable endpoints.

Conclusion

The most sensitive acute QSAR endpoint for fish is 44.28 mg/L for phenols, and the SAR is considered reliable for this metabolite. Due to the solubility of 1.96 mg/L for M750F008 (III CA B.2.14), the predicted toxicities exceed the measured solubility for this metabolite, the solubility of the metabolite itself should be used as a surrogate endpoint. This has been done for fish, *Daphnia* and algae. For both *Daphnia* and algae, the predicted value was in line with the toxicity of the measured values, although it is noted that the studies tested concentrations which exceeded the experimental solubility. Overall, this leads to some uncertainty in the fish endpoint. The QSAR endpoint of M750F008 for fish was four fold less toxic than the toxicity of the parent. In conclusion M750F008 is not expected to be more toxic than the active substance.

Table B.9.2-28: Comparison of endpoints available for M750F008

Substance	QSAR predicted endpoints (mg/L)		
	Fish acute (96-hours)	*Daphnia* acute (48-hours)	Algae (96 hours)
M750F008 (QSAR predicted)	>1.96[1]	>1.96[1]	>1.96[1]
M750F008 (measured)	-	> 8.07	4.08
BAS 750 F (measured)	0.532	0.944	0.679

[1] Adjusted to surrogate toxicity based on the maximum measured solubility of the metabolite

B.9.13. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.9.1.1.1/1	[redacted]	2014a	BAS 750 F-Acute toxicity in the bobwhite quail (Colinus virginianus) after single administration (LD_{50}) 2014/1095701 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.1.1.1/2	[redacted]	2014b	BAS 750 F-Acute toxicity in the mallard duck (Anas platyrhynchos) after single oral administration (LD_{50}) 2014/1095700 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.1.1.1/3	[redacted]	2015a	BAS 750 F-Acute toxicity in the canary (Serinus canaria) after single oral administration (LD_{50}) 2015/1085493 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.1.1.2/1	[redacted]	2014c	BAS 750 F-Avian dietary toxicity test in chicks of the bobwhite quail (Colinus virginianus)	Yes	Yes	Data for first Approval	BASF

			2014/1127963 [redacted] yes Unpublished				
B.9.1.1.2/1a	[redacted]	2015a	Amendment No. 1-BAS 750 F-Avian dietary toxicity test in chicks of the bobwhite quail (Colinus virginianus) 2015/1223324 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.1.1.2/2	[redacted]	2014d	BAS 750 F-Avian dietary toxicity test in ducklings of the mallard duck (Anas platyrhynchos) 2014/1117035 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.1.1.3/1	[redacted]	2014a	BAS 750 F: A reproduction study with the Northern bobwhite 2013/1281276 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.1.1.3/2	[redacted]	2015a	BAS 750 F: A reproduction study with the mallard	Yes	Yes	Data for first Approval	BASF

			2015/7005819 [redacted] yes Unpublished				
B.9.2.1.1/1	[redacted]	2014a	BAS 750 F-Acute toxicity study in the rainbow trout (Oncorhynchus mykiss) 2014/1036951 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.2.1.1/2	[redacted]	2015c	BAS 750 F-Acute toxicity study in the common carp (Cyprinus carpio) 2015/1249071 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.2.1.1/3	[redacted]	2015a	BAS 750 F (Reg.No. 5834378)-Zebrafish acute toxicity test 2015/1001581 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.2.1.1/4	[redacted]	2014a	BAS 750 F: Acute toxicity to the sheepshead minnow, Cyprinodon variegatus, determined under static-renewal test conditions 2014/7002810	Yes	Yes	Data for first Approval	BASF

			[redacted] yes Unpublished				
B.9.2.1.2/1	[redacted]	2015b	Reg.No. 6003432 (metabolite of BAS 750 F, M750F007)-Rainbow trout, acute toxicity test 2015/1001489 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.2.2/1	[redacted]	2015a	BAS 750 F: Early life-stage toxicity test with the sheepshead minnow, Cyprinodon variegatus, under flow-through conditions 2015/7000619 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.2.2/2	[redacted]	2015a	BAS 750 F-Early life-stage toxicity test on the zebrafish (Danio rerio) in a flow through system 2014/1262160 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.2.3/1	[redacted]	2015b	BAS 750 F-Fish sexual development test on the zebrafish (Danio rerio)	Yes	Yes	Data for first Approval	BASF

			2015/1099093 [redacted] yes Unpublished				
B.9.2.3/1a	Obermann M.	2014a	Concentration control analysis of BAS 750 F, Reg.No. 5834378 in mixing-water, GV/T Project No. 56F0741/11E177 2014/1161851 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.3/1b	Obermann M.	2015a	Report Amendment No. 1- Concentration control analysis of BAS 750 F, Reg.No. 5834378 in mixing-water, GV/T Project No. 56F0741/11E177 2015/1117846 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.3/1c	Obermann M.	2015b	Report amendment no. 2 to final report: Concentration control analysis of BAS 750 F, Reg.No. 5834378 in mixing-water, GV/T Project No. 56F0741/11E177 2015/1181295 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF

B.9.2.2/3	[redacted]	2015a	14C-BAS 750 F (label: triazole-3(5)-C14)-Bioconcentration study in the rainbow trout (Oncorhynchus mykiss) 2015/1122811 [redacted] yes Unpublished	Yes	Yes	Data for first Approval	BASF
B.9.2.4.1/1	Brzozowska K.	2014a	BAS 750 F (Reg.No. 5834378)-Daphnia magna, acute immobilization test 2013/1250866 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.4.1/2	VanHooser A.	2014a	BAS 750 F: Acute toxicity test with the saltwater mysid, Americamysis bahia, determined under flow-through test conditions 2014/7002845 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.4.1/3	VanHooser A.	2015a	BAS 750 F: Effect on new shell growth of the eastern oyster (Crassostrea virginica) 2015/7000021 ABC Laboratories Inc., Columbia MO, United States of America yes	No	Yes	Data for first Approval	BASF

			Unpublished				
B.9.2.4.2/1	Backfisch K. Haerthe N.	2015a	Acute toxicity of Reg.No. 6003432 (M750F007; metabolite of BAS 750 F) to Daphnia magna STRAUS in a 48 hour static test 2015/1003915 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.4.2/2	Rzodeczko H.	2015c	Reg.No. 5863469 (metabolite of BAS 750 F, M750F006)-Daphnia magna, acute immobilization test 2015/1001492 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.4.2/3	Rzodeczko H.	2015d	Reg.No. 6003433 (metabolite of BAS 750 F, M750F005)-Daphnia magna, acute immobilization test 2015/1001490 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.4.2/4	Rzodeczko H.	2015e	Reg.No. 6010286 (metabolite of BAS 750 F, M750F008)-Daphnia magna, acute immobilization test 2015/1001493 Institute of Industrial Organic Chemistry,	No	Yes	Data for first Approval	BASF

			Pszczyna, Poland yes Unpublished				
B.9.2.5/1	Janson G.-M.	2014a	Chronic toxicity of the BAS 750 F (Reg.No. 5834378) to Daphnia magna STRAUS in a 21 day semi-static test 2014/1098028 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.5/2	Janson G.-M.	2015b	Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1003912 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.5/2a	Janson G.-M.	2015c	Report Amendment No.1-Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia longispina in a 21 day semi-static test 2015/1251197 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.5/3	Janson G.-M.	2015a	Chronic toxicity of BAS 750 F (Reg.No. 5834378) to Daphnia pulex in a 21 day semi-static test 2015/1003913 BASF SE, Limburgerhof, Germany Fed.Rep.	No	Yes	Data for first Approval	BASF

			yes Unpublished				
B.9.2.5/4	Dinehart S.	2016a	BAS 750 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through conditions 2016/7001293 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.5/5	Clark R.	2015a	BAS 750 F-10-day toxicity test exposing midge (Chironomus dilutus) to a test substance applied to sediment under static-renewal conditions 2015/7000621 Smithers Viscient LLC, Wareham MA, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.5/6	Backfisch K. Weltje L.	2015a	Chronic toxicity of Reg.No. 5924326 (M750F003; metabolite of BAS 750 F) to the non-biting midge Chironomus riparius-a spiked sediment study 2015/1003916 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.5/7	Backfisch K. Weltje L.	2015a	Chronic toxicity of Reg.No. 5834378 to the non-biting midge Chironomus riparius-A	No	Yes	Data for first Approval	BASF

			spiked sediment study 2014/1243181 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished				
B.9.2.5/8	Clark R.	2015b	BAS 750 F-10-Day toxicity test exposing freshwater amphipods (Hyalella azteca) to a test substance applied to sediment under static-renewal conditions 2015/7000622 Smithers Viscient LLC, Wareham MA, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.5/9	Clark R.	2015c	BAS 750 F-10-Day toxicity test exposing estuarine amphipods (Leptocheirus plumulosus) to a test substance applied to sediment under static conditions 2015/7000623 Smithers Viscient LLC, Wareham MA, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.7.1/1	Brzozowska K.	2014b	BAS 750 F (Reg.No. 5834378)-Pseudokirchneriella subcapitata SAG 61.81-Growth inhibition test 2013/1250865 Institute of Industrial Organic Chemistry, Pszczyna, Poland	No	Yes	Data for first Approval	BASF

			yes Unpublished				
B.9.2.7.1/2	Bergfield A.	2015a	BAS 750 F: Growth inhibition test with the marine diatom, Skeletonema costatum 2015/7000620 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.7.1/3	Bergfield A.	2015b	BAS 750 F: Growth inhibition test with the freshwater diatom, Navicula pelliculosa 2015/7000618 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.7.1/4	Bergfield A.	2015c	BAS 750 F: Growth inhibition test with the cyanobacterium, Anabaena flos-aquae 2015/7000617 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.7.2/1	Backfisch K.	2015a	Effect of Reg.No. 6003432 (M750F007, metabolite of BAS 750 F) on the growth of the green alga Pseudokirchneriella subcapitata 2015/1003914 BASF SE, Limburgerhof,	No	Yes	Data for first Approval	BASF

			Germany Fed.Rep. yes Unpublished				
B.9.2.7.2/2	Brzozowska-Wojoczek K.	2015a	Reg.No. 6010286 (metabolite of BAS 750 F, M750F008)-Pseudokirchneriella subcapitata SAG 61.81-Growth inhibition test 2015/1001491 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.7.2/3	Rzodeczko H.	2016a	Reg.No. 5863469 (metabolite of BAS 750 F, M750F006)-Pseudokirchneriella subcapitata SAG 61.81-Growth inhibition test 2015/1184815 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.7.2/4	Rzodeczko H.	2016b	Reg.No. 6003433 (metabolite of BAS 750 F, M750F005)-Pseudokirchneriella subcapitata SAG 61.81-Growth inhibition test 2015/1184816 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.2.8/1	Swierkot A.	2014a	BAS 750 F (Reg.No. 5834378)-Lemna gibba CPCC 310 growth	No	Yes	Data for first Approval	BASF

			ihibition test 2014/1001322 Institute of Industrial Organic Chemistry, Pszczyna, Poland yes Unpublished				
B.9.3/1	Franke M.	2015a	Acute toxicity of BAS 750 F to the honeybee Apis mellifera L. under laboratory conditions 2015/1128674 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.3/2	Amsel K.	2015a	Acute toxicity of BAS 750 F to the bumblebee Bombus terrestris L. under laboratory conditions 2014/1275250 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.3/3	Franke M.	2015b	Acute toxicity of BAS 750 F to the honeybee Apis mellifera L. under laboratory conditions 2015/1128674 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep.	No	Yes	Data for first Approval	BASF

			yes Unpublished				
B.9.3/4	Amsel K.	2015a	Acute toxicity of BAS 750 F to the bumblebee Bombus terrestris L. under laboratory conditions 2014/1275250 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.3/5	Kleebaum K.	2015a	Chronic toxicity of BAS 750 F (Reg.No. 5834378) to the honeybee Apis mellifera L. under laboratory conditions 2013/1235086 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.3/6	Kleebaum K.	2015b	Acute toxicity of BAS 750 F to honeybee larvae (Apis mellifera L.) under laboratory conditions (in vitro) 2013/1235087 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.3/7	Royer S.	2015a	BAS 750 F (Reg.No. 5834378)-Honey bee larvae test (repeated	No	No	Not applicable	BASF

			exposure, observation 21 days) under laboratory conditions (in vitro)-Non-GLP 2014/1327676 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished				
B.9.4.1/1	Friedrich S.	2013a	Sublethal toxicity of Reg.No. 5834378 (BAS 750 F) to the earthworm Eisenia fetida in artificial soil 2013/1235075 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.4.2/1	Friedrich S.	2013b	Effects of BAS 750 F on the reproduction of the collembolan Folsomia candida 2013/1235081 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.4.2/2	Schulz L.	2014a	Effects of BAS 750 F on the reproduction of the predatory mite Hypoaspis aculeifer 2013/1235082 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany	No	Yes	Data for first Approval	BASF

			Fed.Rep. yes Unpublished				
B.9.4.2/3	Schulz L.	2014b	1,2,4-triazole-CGA71019-Effects on the reproduction of the predatory mite Hypoaspis aculeifer 2014/1326895 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	TDMG
B.9.5/1	Schulz L.	2015a	Effects of BAS 750 F (Reg.No. 5834378) on the activity of soil microflora (Nitrogen transformation test) 2015/1108623 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.7/1	Friedrich S.	2015a	Acute toxicity of BAS 750 F to the earthworm Eisenia fetida in artificial soil with 10% peat 2015/1003342 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF
B.9.7/2	Schulz L.	2015b	Effects of BAS 750 F (Reg.No. 5834378) on the	No	Yes	Data for first	BASF

			activity of soil microflora (Carbon transformation test) 2015/1108621 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished			Approval	
B.9.8/1	Hammer S.	2014a	BAS 750 F-Determination of the inhibition of oxygen consumption in the activated sludge respiration inhibition test 2014/1049095 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	Data for first Approval	BASF